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RESEARCH PAPER

# A Uniform Ultra-Small Microsphere/SAIB Hybrid Depot with Low Burst Release for Long-Term Continuous Drug Release

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## ABSTRACT

***Purpose*** In the present study, a uniform ultra-small microsphere/sucrose acetate isobutyrate (SAIB) hybrid depot (m-SAIB depot) was designed to provide a long-term sustained release drug delivery system which not only reduced the burst release of an SAIB depot, but also eliminated the lag-time of PLGA microspheres.

***Methods*** Risperidone loaded m-SAIB depot (Ris-m-SAIB depot) was characterized by *in vitro* drug release, pharmacokinetics, *in vivo* degradation and biocompatibility, in comparison with risperidone loaded SAIB depot (Ris-SAIB depot).

***Results*** Ris-m-SAIB depot showed a low burst release (0.64%) and a reduced *in vitro* drug release rate due to the encapsulation of most drug in microspheres. After intramuscular administration, the *in vivo* burst release of Ris-m-SAIB was significantly decreased, as reflected by the low $C_{max}/C_{s(4\text{-}td)}$ (approximately 30-fold reduction), in comparison with Ris-SAIB depot. From 4 to 78 days, Ris-m-SAIB depot showed a higher plasma drug level (1.55~16.30 ng/ml) with a steadier drug release profile compared with Ris-SAIB depot. Ris-m-SAIB depot degraded gradually with a degradation $t_{1/2}$ of 54.6 days and exhibited good biocompatibility *in vivo*.

***Conclusion*** These results demonstrate the potential application of a uniform ultra-small microsphere/SAIB hybrid depot for continuously delivering small drug molecules for long periods of time without burst release.

**KEY WORDS** uniform ultra-small microsphere/SAIB hybrid depot · burst release · continuous drug release · degradation *in vivo* · biocompatibility

## ABBREVIATIONS

| | |
|---|---|
| 9-OH-Ris | 9-Hydroxyrisperidone |
| AUC | Area under the curve |
| BA | Benzyl alcohol |
| $C_{max}$ | Maximum concentration |
| $C_{min}$ | Minimum concentration |
| $C_s$ | Mean value of plasma concentration at steady-state |
| DAS | Drug and statistics software |
| DCM | Dichloromethane |
| EtOH | Ethanol |
| HPLC | High performance liquid chromatography |
| $Mg(OH)_2$ | Magnesium hydroxide |
| PBS | Phosphate buffered saline |
| PLA | Poly-lactide |
| PLGA | Poly (lactide-coglycolide) |
| PVA | Poly (vinyl alcohol) |
| Ris | Risperidone |
| Ris-m-SAIB depot | Risperidone loaded microsphere/SAIB hybrid depot |
| Ris-SAIB depot | Risperidone loaded SAIB depot |
| SAIB | Sucrose acetate isobutyrate |
| SEM | Scanning electron microscopy |

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| | |
|---|---|
| SPG | membrane Shirasu porous glass membrane |
| $t_{1/2}$ | Half-life |
| UPLC–MS/MS | Ultra performance liquid chromatography-tandem mass spectrometry |

## INTRODUCTION

Since an increasing number of diseases, such as schizophrenia, hormone-dependent conditions, and diabetes, require long-term drug treatment, sustained-release drug delivery systems that can exhibit long periods of drug release have attracted much attention (1–3). Poly(lactide-co-glycolide) (PLGA)-based microspheres have been widely applied to deliver synthetic small molecules and large molecules, due to the excellent degradability and biocompatibility of PLGA (4–8). However, PLGA microspheres usually display tri-phasic drug release profiles *in vitro* and *in vivo*, characterized by an initial burst release followed by a lag-time of several days or weeks with little drug release and finally a rapid drug release (9–11). Therefore, after the injection of PLGA microspheres, such as commercial products RISPERDAL® CONSTA®, due to the drug release lag-time, oral drug supplementation should be given during the lag-time phase to maintain therapeutic concentrations until the drug begins to be released again (12, 13). In addition, for such PLGA microspheres, in order to reach steady plasma concentrations, four injections in 8 weeks were required. It is therefore essential to improve the tri-phasic drug release behavior of PLGA microspheres to provide continuous release without a lag-release phase to maintain the therapeutic effect of the drug in PLGA microspheres. To date, many efforts have been made to eliminate the lag-phase and thus obtain continuous drug release: (1) increasing the water penetration by co-encapsulation of a pore agent in microspheres such as $Mg(OH)_2$ (6), stearic acid (14), and medium chain triglyceride (15), (2) improving the hydrophilic nature of PLGA by using low molecular weight PLGA (15–17), (3) and controlling the drug release by diffusion by preparing small-sized microspheres (e.g., smaller than 20 μm for small molecules) (18, 19). Although the above approaches all successfully eliminate the lag-release phase by increasing the drug diffusion rate during the initial stage, problems, such as burst release and a significantly shortened drug release period caused by those approaches, have occurred. Therefore, it is very difficult to develop a long-term sustained-release drug delivery system with an ideal drug release profile using PLGA microspheres alone.

Another interesting injectable sustained-release system is a sucrose acetate isobutyrate (SAIB) *in situ* depot that is a non-polymeric material with high viscosity and hydrophobicity. An attractive property of this SAIB depot is that it is possible to tailor the drug release period of the SAIB depot from a few hours to several months. In addition, the viscosity of the SAIB/solvent can be dramatically reduced to 0.05 ~ 0.2 Pa·s by inclusion of a minimal amount of solvent, e.g., 10 ~ 15% ethanol, allowing the SAIB/solvent to be easily injected using small needles. Upon injection, the solvent diffuses from the depot into body fluid, forming a highly viscous SAIB depot which can release drug slowly for long period of time. Due to the good syringe-related characteristics, tailorable drug release period, and the large loading capacity for various drugs, the SAIB depot has been widely applied to deliver various drugs, such as small molecules (20, 21), polysaccharides (22), peptides (23), and proteins (24). Despite the ability to provide sustained drug release for months, an initial burst release always occurs with all drugs independent of the physicochemical properties of the drug, which is caused by solvent diffusion druing the depot formation. Therefore, how to effectively reduce the burst release is very important for the development of an SAIB depot and considerable efforts have been made to reduce the burst release of SAIB depots. Biodegradable polymers, including PLA and PLGA, have been dissolved in SAIB/solvent systems to reduce the burst release (25). The burst release was reduced *in vitro* due to the decreased mass transfer rate (i.e., the diffusion rate of drug in the depot) after inclusion 10% (*w*/*w*) of PLA or PLGA in SAIB. However, after injecting the formulation into rats, the initial burst release remains *in vivo* (25, 26). This indicates that simple reduction of the mass transfer rate may not be effective to reduce burst release from the SAIB depot *in vivo*, although sometimes this approach is useful in reducing the burst release *in vitro*. It is therefore necessary to investigate the main factor that causes the *in vivo* burst release. During the *in vivo* formation of the SAIB depot, the diffusion of solvent from SAIB into the surrounding tissue occurs first, during which the dissolved drug in the solvent is prone to migrate with the solvent, resulting in high amount of drug being released into the surrounding tissue and a high drug concentration at the interface between the formed depot and the tissue (27). Consequently, the migration of solvent containing dissolved drug makes the main contribution to the burst release, while the relatively high drug concentration at the interface contributes to the subsequent rapid release. Therefore, it is highly likely that reducing the amount of dissolved drug in the solvent in SAIB might be effective in reducing the *in vivo* burst release and, hence, allow SAIB to provide long-term sustained drug release.

Ideally, a long term sustained-release drug delivery system should be capable of delivering drug molecules continuously and steadily for a sufficiently long period of time without any burst release. By taking advantages of PLGA and SAIB in terms of controlling drug release as mentioned above, in this study we designed a uniform ultra-small microsphere/SAIB hybrid depot, as illustrated in Fig. 1, to achieve such a drug delivery system. Basically, we prepared ultra-small drug-

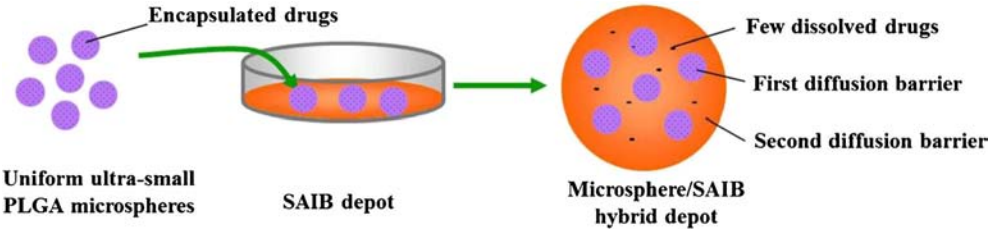


**Fig. 1** The schematic diagram of the uniform ultra-small microsphere/SAIB hybrid depot.

loaded microspheres using PLGA and then dispersed the microspheres evenly in SAIB. Uniform ultra-small PLGA microspheres (<10 μm) were prepared with a low molecular weight PLGA (28 KDa) to entrap most of drug with the expectation of releasing drug continuously via the diffusion-controlled process. Moreover, the small sized microspheres would allow the drug to be evenly distributed in the final hybrid depot. The uniform ultra-small PLGA microspheres were then dispersed in an SAIB/solvent system uniformly, during which only the drug at the surface of the PLGA microspheres can be dissolved in the SAIB/solvent system. Therefore, after injection, only the dissolved drug from the surface of the microspheres may be released, resulting in a low initial drug release of SAIB depot and, hence, reducing the burst release of SAIB. After formation of the depot, drug molecules are blocked by double diffusion barriers whereby the drug must diffuse through microspheres to the SAIB depot first and then diffuse from the SAIB depot into the media, leading to a steady and slow drug release profile. Meanwhile, due to the application of low molecular weight PLGA and the decreased particle size (<10 μm), the ultra-small microspheres will ensure continuous and complete drug release as reported in the literature (15–19). As a result, the microsphere/SAIB hybrid depot can not only reduce the burst release of SAIB but also significantly prolong the drug release period of microspheres prepared with low molecular weight PLGA, rendering the hybrid depot a useful and practical long-term drug delivery system.

Risperidone (Ris), a hydrophobic drug, has a high affinity for SAIB. The equilibrium solubility of Ris in SAIB/EtOH (85/15, *w*/*w*) is approximately 20 mg/ml (28) and it has been reported that by dispersing Ris in SAIB depot, the drug release can be extended to 20 days after intramuscular administration (26). Initial burst release, however, was still observed both *in vitro* and *in vivo*, which was mainly caused by the high solubility of Ris in the SAIB depot. Moreover, as mentioned previously, the Ris-loaded PLGA microspheres (RISPERDAL® CONSTA®) exhibit typical tri-phasic drug release behavior with a 3-week lag-time. Therefore, in order to assess the designed microsphere/SAIB hybrid depot, risperidone was selected as the model small drug molecule.

In addition, an SAIB-based depot has been successfully applied to deliver bupivacaine (POSIDUR™) for over 72 h, and a Phase III clinical trial has been completed. FDA stated that the new drug application should provide sufficient information to demonstrate the safety of POSIDUR™ after injection (29). However, to the authors' knowledge, there is little published data on the safety of the SAIB-based depot after intramuscular injection. In this study, in addition to developing a non-burst release hybrid depot system, a safety study, including *in vivo* degradation and biocompatibility, of the SAIB-based hybrid depot was also conducted, and this is the very first report about the degradation of the SAIB-based depot after intramuscular injection.

Overall, in this study, we intended to develop a novel hybrid drug delivery system by dispersing ultra-small microspheres in SAIB to release the drug continuously and steadily for a sufficiently long period of time without burst release. The system also solved the common problem of burst release when using SAIB and eliminated the drug release time lag when using PLGA microspheres.

## MATERIALS AND METHODS

SAIB was purchased from Sigma Aldrich (density of 1.146 g/ml at 25°C, St. Louis, MO). Poly (lactide-coglycolide) (PLGA) 75/25 (28 kDa, inherent viscosity of 0.54 dl/g in $CHCl_3$ at 25°C) was supplied by Changchun Institute of Applied Chemistry (Changchun, China). Risperidone (Ris) was provided by Jinan Hui Feng Da Chemical CO., LTD. (Jinan, China). Poly (vinyl alcohol) (PVA, 87−89% hydrolyzed, average Mw = 72,600–81,400) was obtained from Kuraray CO., LTD. (Osaka, Japan). Benzyl alcohol (BA), dichloromethane (DCM) and ethanol (EtOH) were purchased from Concord Technology CO., LTD. (Tianjin, China). All other chemicals and solvents were of chromatographic grade.

### Preparation of Ris-microspheres by the Membrane Emulsification Technique

Four hundred milligrams PLGA and 200 mg RIS were dissolved in 2 ml organic solvent (DCM/BA, 8/2, *v*/*v*). The solution was then injected into 20 ml water containing 1% PVA (*w*/*v*) under stirring at 3000 rpm for 30 s using an Ultra Rurrax (IKA T18 basic, Germany), at a constant temperature of 8°C to produce a coarse emulsion. The obtained emulsion was poured immediately into a membrane

emulsification apparatus (Mini kit, SPG TECHNOLOGY CO., Ltd, Japan) fitted with a Shirasu porous glass (SPG) membrane (10.0 μm pore diameter SPG TECHNOLOGY CO., Ltd, Japan). The SPG membrane was soaked in a continuous phase (1% PVA solution) and sonicated for 30 min prior to homogenization. The coarse emulsion was forced to pass through the SPG membrane at 0.05 MPa under the pressure of nitrogen gas. The resulting emulsion was then poured into 180 ml 1% PVA solution (*w*/*v*) to extract BA. The residual organic solvent was removed at 40°C under reduced pressure. Ris-microspheres were collected by centrifugation at 3000 rpm for 10 min, washed three times with distilled water, and then freeze-dried (VirTis AdVantage Plus Bench Top Freeze Dryer, SP Industries, Inc., USA).

The particle size distribution of Ris-microspheres was investigated by a Beckman Coulter LS230 Laser Diffraction Particle Analyzer (Beckman-Coulter Inc., USA). The microspheres were re-dispersed in distilled water containing 0.1% PVA (*w*/*v*) prior to analysis.

The morphology of the Ris-microspheres was examined using scanning electron microscopy (SEM) (Shimadzu SSX-500, Tokyo, Japan). Freeze-dried Ris-microspheres were spread on double-sided conductive adhesive tape, which was previously attached to a copper stub. The sample was then coated with a gold layer and observed by SEM.

Ris-microspheres were dissolved in acetonitrile and sonicated for 15 min. The concentration of Ris was determined by HPLC (28). Drug loading was calculated from the weight of drug *versus* the weight of microspheres. Encapsulation efficiency was calculated from the amount of Ris entrapped in microspheres *versus* the total amount of drug used for microsphere preparation.

## Preparation of Ris-loaded Uniform Ultra-Small Microsphere/SAIB Hybrid Depot (Ris-m-SAIB Depot)

Prior to use, a certain amount of Ris-microspheres was suspended in the SAIB/EtOH (85/15, *w*/*w*) system by vortexing for 5 min. The prepared Ris-m-SAIB depot had a final drug loading of 25 mg/g.

## Preparation of Bare Ris-loaded SAIB Depot (Ris-SAIB Depot)

Ris-SAIB depot was prepared as previously reported (28). Ris was dissolved in 1% acetic acid solution and spray-dried using a spray drier (EYELA SD 1000, Japan). The resulting powder was then dispersed in an SAIB/EtOH (85/15, *w*/*w*) system and sonicated until dispersed uniformly. The prepared Ris-SAIB depot had a final drug loading of 25 mg/g.

## *In Vitro* Release Studies

About 0.1 g Ris-m-SAIB depot and Ris-SAIB depot (equal to 2.5 mg Ris) was respectively injected into 3 ml 10 mM phosphate buffered saline (PBS) solution (pH 7.4, 0.02% $NaN_3$) in an EP tube. Similarly, a certain amount of Ris-microspheres (equal to 2.5 mg Ris) was dispersed in the above PBS solution. All the samples were incubated in a ZWY-110X30 reciprocal shaking water bath (Zhicheng Inc., China) at 37°C. Samples were removed at every predetermined time point and replaced with 3 ml fresh release medium. The samples were analyzed using HPLC. All experiments were carried out in triplicate.

## Pharmacokinetic Study

The pharmacokinetics of the Ris-m-SAIB depot and Ris-SAIB was compared. Male Wistar rats, with the weight of 180–220 g, were obtained from the Experimental Animal Center of Shenyang Pharmaceutical University (Shenyang, China). After 7 days acclimatization, the rats were divided into two groups (six rats per group). Animals were all fasted for 12 h with access to water *ad libitum* prior to dosing. For one group, Ris-m-SAIB depot was intramuscularly injected into the right hind-leg muscle as a single dose of 12.5 mg/kg. For the other group, Ris-SAIB depot was administered in a similar manner. Blood samples, approximately 0.3 ml, were collected via retro-orbital puncture and transferred to heparinized tubes at 2, 4, 6, 8 and 12 h, and 1, 2, 4, 6, 8, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 50, 54, 65 and 78 days. The heparinized blood samples were subjected to centrifugation at 4000 rpm for 10 min and the plasma samples obtained were collected and stored at −80°C until subsequent extraction and analysis. The plasma concentration of Ris and its active metabolite (9-OH-Ris) were determined using ultra performance liquid chromatography-tandem mass spectrometry (UPLC–MS/MS, Waters Corp., Milford, MA). The obtained data were analyzed using a non-compartmental method with drug and statistics (DAS) software (version 2.0, Mathematical Pharmacology Professional Committee of China, Shanghai, China).

## *In Vivo* Degradation and Biocompatibility Study

Male Wistar rats (180–220 g, Experimental Animal Center of Shenyang Pharmaceutical University, Shenyang, China) received an intramuscular injection of Ris-m-SAIB depot in the right hind-leg muscle as a single dose of 12.5 mg/kg (about 0.1 g depot). The mass of depot injected was recorded (calculated by reducing weight method). Three rats were sacrificed 1, 2, 4, 6, 10, 14, 18, 22, 26, 30, 54 and 78 days after administration. The remaining depot, injected into the right hind-leg muscle, was retrieved. After removal of surrounding tissue, the

remaining depot was freeze-dried and weighed. The mass remaining (%) was calculated from the following equation:

$$\text{Mass remaining (\%)} = \text{The weight of remaining depot/The total weight of injected depot} \times 100\%$$

Tissues surrounding the depot were immediately fixed in formalin, dehydrated, paraffin embedded, sectioned and stained with hematoxylin and eosin. The histological sections were observed under light microscopy.

All animal experiments were approved by the University Ethics Committee and all protocols were in accordance with the guidelines of the Care and Use of Laboratory Animals.

## RESULTS

### Characterization of Ris-microspheres

The traditional emulsification solvent-evaporation method, commonly employed to prepare microspheres, generally results in microspheres with a broad particle size distribution. It is therefore difficult to suspend the polydisperse microspheres in the SAIB depot evenly and closely control the drug release. In this study, the premix membrane emulsification method, a commonly used method for preparing uniform microspheres (30–32), was applied to produce Ris-microspheres with a narrow particle size distribution. The parameters of the preparation process and the formulation were optimized by assessing major factors including burst release (in terms of the cumulative drug release after 1 day), particle size distribution, drug loading, and encapsulation efficiency. The optimized Ris-microspheres had a mean particle size of 5.910±2.243 μm, which was sufficiently small and uniform to allow dispersion in the SAIB depot. The SEM images (Fig. 2) confirmed that the particle size of Ris-microspheres was evenly distributed. Ris-microspheres with a relatively high drug loading (19.97%) and encapsulation efficiency (59.97%) were obtained using solvent extraction combined with vacuum rotary evaporation to remove the solvent. Drug release from the microspheres alone was only 1.14% after 1 day, indicating that most of the drug was encapsulated in the microspheres. This extremely low drug release from the microspheres may significantly reduce the free drug concentration (dissolved drug concentration) in the final Ris-m-SAIB depot and, subsequently, reduce the burst release of depot.

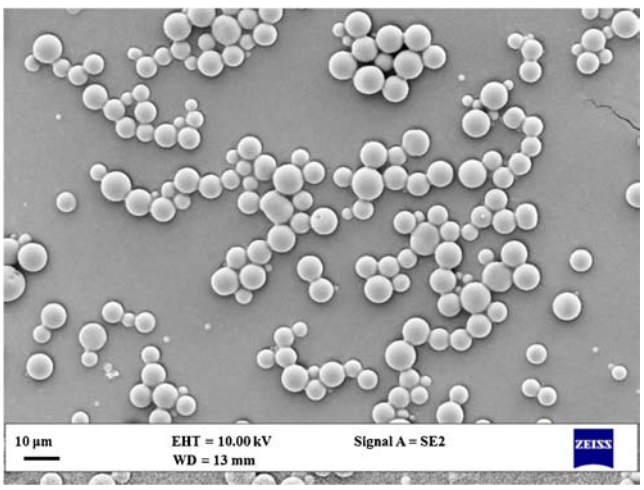


**Fig. 2** Representative SEM images (×2 K) of Ris-microspheres.

### *In Vitro* Drug Release Behavior of Ris-m-SAIB Depot

The *in vitro* drug release profiles of Ris from Ris-microspheres, Ris-SAIB depot and Ris-m-SAIB depot are shown in Fig. 3. As seen in the Fig. 3, approximately 12% of the drug was released from the Ris-SAIB depot in 24 h in PBS medium, indicating a significant burst release for this formulation. No burst release, however, was seen in Ris-microspheres alone where the cumulative drug release was only 1.14% in the same medium after 1 day, suggesting that most of the drug was encapsulated in the microspheres. In the next 28 days, drug release from microspheres occurred at a nearly constant rate, approximately 2.39% per day as seen in Fig. 3. After 60 days, most of drug, up to 81.58%, was released from the microspheres. The continuous and complete drug release from microspheres into the medium implied that the drug could continuously diffuse from the microspheres into the SAIB depot, which thus maintained continuous drug release of the whole hybrid depot. After loading Ris-microspheres into SAIB depot, the burst release was dramatically reduced to 0.64% after 1 day, compared with that of the Ris-SAIB depot (up to 12.16%). The above results indicate that the *in vitro* burst drug release from the SAIB depot was effectively reduced by incorporating Ris-microspheres into SAIB.

The reduced burst release of Ris-m-SAIB depot was mainly attributed to the reduced amount of dissolved drug in the solvent in the depot. Upon the injection of SAIB depot into the medium, the drug was released from the depot into the medium along with the fast diffusion of solvent with the dissolved drug during the depot formation (25). This was the case for Ris-SAIB depot, as reflected by the burst release (up to 12.16%). However, most of the drug in the Ris-m-SAIB depot was incorporated in microspheres. Therefore, only a small amount of drug which may be from the surface of the

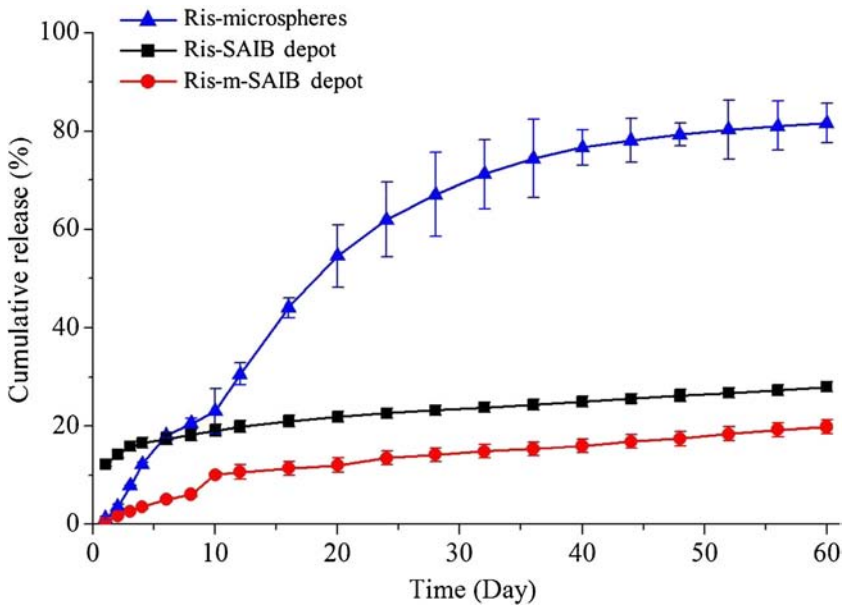


**Fig. 3** *In vitro* drug release profiles of Ris from different formulations (containing 2.5 mg Ris) in release medium (10 mM PBS, pH 7.4, 0.02% NaN3) at 37°C. Each point represents the mean ± S.D.; $n = 3$.

microspheres was released into the medium during the depot formation, leading to a significantly reduced burst release (0.64%). The above results suggested that the burst release from Ris-m-SAIB depot during the formation of depot was restricted by the low drug diffusion rate from the microspheres into the SAIB matrix.

The drug release profiles of Ris-microspheres, Ris-SAIB depot and Ris-m-SAIB depot were all fitted to a Higuchi model using the data without the first day (to avoid the burst release) and acceptable regression coefficients were achieved for all the release profiles (Table I), indicating that the drug release from the three systems was diffusion controlled. This result may also indicate that the drug release from Ris-m-SAIB depot was controlled by double diffusion barriers whereby the drug must dissolve and diffuse from the microspheres into the SAIB matrix and then diffused from the SAIB matrix into the medium.

From day 1 to day 8 after injection into the medium, the drug release rate of Ris-m-SAIB depot was slightly slower than that of Ris-SAIB depot, as reflected by the smaller slope of Ris-m-SAIB depot in comparison with that of Ris-SAIB depot. This indicates that the drug release from the microspheres into the SAIB matrix might be slightly slower than the drug release from the SAIB matrix into the PBS medium. Although the drug release rate of Ris-microspheres from day 1 to day 8 (slope = 11.59, $r = 0.9902$, data was not shown in Table I) was significantly higher than that of Ris-SAIB depot in the same PBS medium, the drug release from the microspheres in the SAIB matrix would be slower than that in the PBS medium since that the viscosity of SAIB matrix (approximately 100 Pa·s) (28) was extremely higher than that of the PBS medium (approximately $0.69 \times 10^{-3}$ Pa·s).After 10 days, the drug release rate of Ris-m-SAIB depot was slightly higher than that of Ris-SAIB depot. This can be attributed to the discrepancy in the remaining drug concentrations after 10 days between the two formulations that about 18% of the drug had been released from Ris-SAIB depot into the medium, whereas only 10% of the drug had been released from Ris-m-SAIB depot and therefore the higher remaining drug concentration in Ris-m-SAIB depot can act as a stronger driving force for the drug release.

The above *in vitro* results indicated that the drug release rate from the microspheres not only influenced the initial burst release but also affected the subsequent drug release rate of Ris-m-SAIB depot. A slow drug release rate from the microspheres could effectively reduce the initial drug release rate of Ris-m-SAIB depot and might slightly increase the subsequent drug release rate.

It should be noted that after 60-day drug release test, the drug release of Ris-SAIB depot and Ris-m-SAIB depot was

**Table I** Evaluation of Drug Release Kinetics of Different Formulations According to the Higuchi Equation

| Formulations | Ris-microspheres | | | Ris-SAIB depot | | | Ris-m-SAIB depot | | |
|---|---|---|---|---|---|---|---|---|---|
| | Burst release (%) | 1–40 day | 40–60 day | Burst release (%) | 1–8 day | 10–60 day | Burst release (%) | 1–8 day | 10–60 day |
| Slope | 1.14 | 15.75 | 3.51 | 12.16 | 3.26 | 1.85 | 0.64 | 3.05 | 2.16 |
| R | | 0.9919 | 0.9910 | | 0.9702 | 0.9977 | | 0.9989 | 0.9946 |

both below 30% (28.04% for Ris-SAIB depot and 19.89% for Ris-m-SAIB depot). In order to ensure that there was no recovery issue of the dissolution test method, HPLC was applied to determine the remaining amount of the drug in the formulation, and it was shown that circa 71.05 and 78.6% of the total drug was still locked in the residual Ris-SAIB depot and Ris-m-SAIB depot respectively after a 60-days *in vitro* drug release assessment. The drug locked in the depot could not be released completely under the *in vitro* condition. This was mainly because that the SAIB was non-degradable *in vitro*, as reflected by the constant weight of the depot. Therefore, it is essential to further conduct an *in vivo* evaluation on the SAIB-based depots.

### *In Vivo* Pharmacokinetics of Ris-m-SAIB Depot

The *in vitro* drug release study indicates that the initial burst release and subsequent release of Ris-SAIB depot was significantly reduced by the incorporation of drug into microspheres prior to loading drug in SAIB depot. To further evaluate the *in vivo* drug release behavior, a comparative *in vivo* pharmacokinetic study of the two depots was conducted. As microspheres smaller than 10 μm can undergo phagocytosis by macrophages after intramuscular administration (33, 34), no *in vivo* pharmacokinetics study of Ris-microspheres was conducted.

The plasma concentration-time profiles of Ris, 9-OH-Ris (the major active metabolite of Ris) and the total active components (Ris plus 9-OH-Ris) after intramuscular administration of different SAIB depots at a single dose of 12.5 mg/kg are shown in Figs. 4, 5 and 6, respectively.

In the first 4 days after administration, the Ris, 9-OH-Ris and total active components plasma concentration levels of Ris-m-SAIB depot were all much lower than those of Ris-SAIB depot. For the Ris-SAIB depot, the $C_{max}$ of Ris, 9-OH-Ris and the total active components was 821.4±347.3, 536.9±229.0 and 1283.2±455.3 ng/ml respectively, while the corresponding $AUC_{0\text{-}4d}$ values were 556.0±142.0, 440.1±111.3 and 996.1±190.4 ng/ml·d. By loading Ris-microspheres into SAIB depot, the $C_{max}$ of Ris, 9-OH-Ris and the total active components were significantly reduced to 40.8±13.7, 34.4±16.9 and 73.9±30.6 ng/ml respectively, while the corresponding $AUC_{0\text{-}4d}$ values were reduced to 66.7 ±9.1, 38.6±16.1 and 105.3±24.4 ng/ml·d, which is approximately a 10-fold reduction ($p<0.05$). The above results demonstrate that the Ris-m-SAIB depot exhibited a low burst release.

Then, 4 days after administration, the risperidone and 9-OH-risperidone plasma concentrations of Ris-SAIB depot both dropped to below 10 ng/ml (Figs. 4 and 5). After 22 days, the risperidone plasma concentrations were below the lowest limit of quantification of the analytical method (LLOQ, 0.1 ng/ml). The 9-OH-risperidone plasma concentration dropped below the LLOQ (0.1 ng/ml) after 14 days. For the Ris-m-SAIB depot, the plasma concentration of both risperidone and 9-OH-risperidone can still be detected until 78 days after administration. The total active components plasma concentrations from 4 to 78 days were in the range of 1.55~16.30 ng/ml, which was higher than those of risperidone-loaded SAIB depot (Fig. 6). These results suggest that drug release can be prolonged by loading Ris-microspheres into SAIB depot.

Moreover, the $C_{max}/C_{s(4\text{-}t\ d)}$ of the two depots (with and without the incorporation of microspheres) was calculated to further evaluate the effect of loading microspheres into SAIB depots on reducing burst release, where $C_{max}$ is the maximum plasma concentration, $C_{s(4\text{-}t\ d)}$ represents the mean plasma concentration from 4 to 78 days and a large $C_{max}/C_{s(4\text{-}t\ d)}$

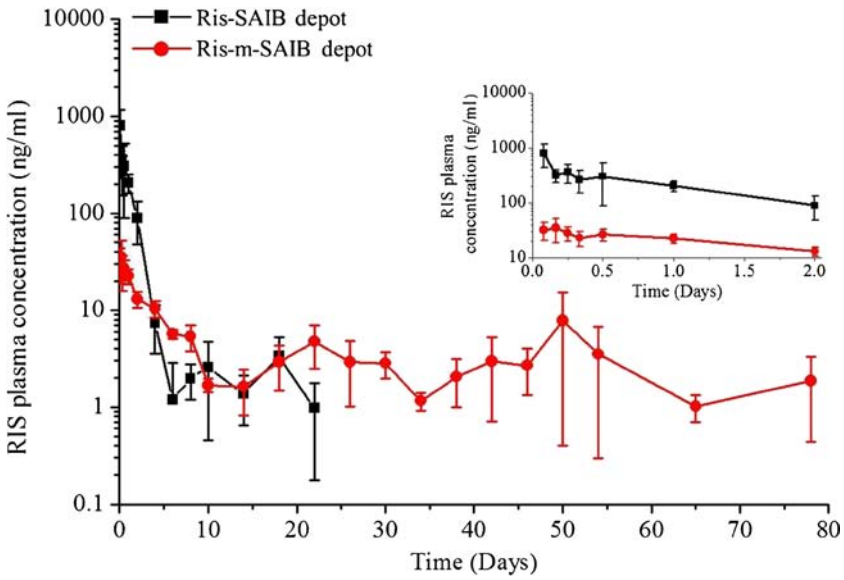


**Fig. 4** Plasma concentration-time profiles of Ris after intramuscular injection of Ris-SAIB depot and Ris-m-SAIB depot (with a drug loading of 25 mg/g) to rats at a dose of 12.5 mg/kg (mean ± SD, $n = 6$).

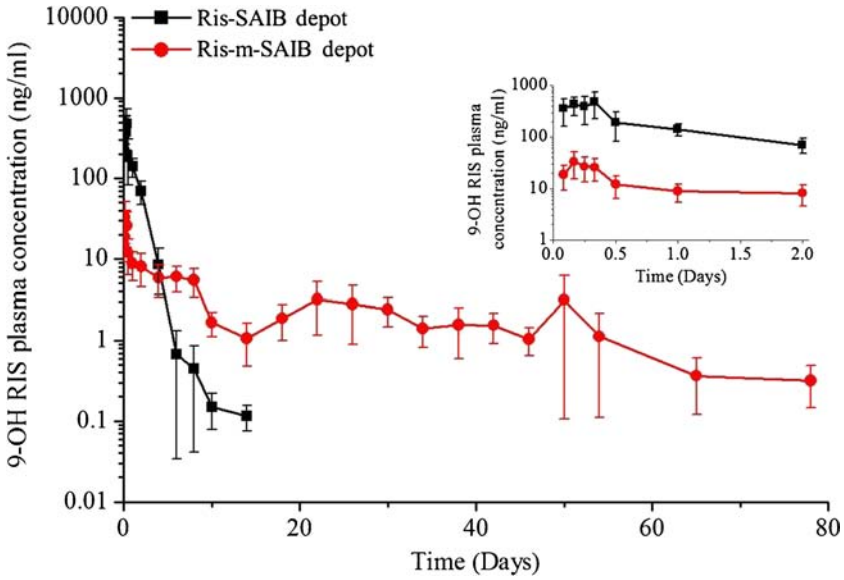


**Fig. 5** Plasma concentration-time profiles of 9-OH-Ris (an active metabolite of Ris) after intramuscular injection of Ris-SAIB depot and Ris-m-SAIB depot (with a drug loading of 25 mg/g) to rats at a dose of 12.5 mg/kg (mean ± SD, $n = 6$).

value represents a high burst release. As shown in Table II, the $C_{max}/C_s$ value of Ris was significantly reduced from 291.1 ± 94.4 to 11.3 ± 3.1 after loading Ris-microspheres into SAIB depot compared with that of Ris-SAIB depot ($p < 0.05$). Similar results were also obtained for 9-OH-Ris and the total active components, as shown in Tables III and IV ($p < 0.05$). These results also confirmed that encapsulating Ris into microspheres could significantly reduce the burst release of SAIB depot, which was in agreement with the results of the *in vitro* drug release study.

The *in vivo* release behaviors after 4 days of administration were further assessed using $C_{max(4\text{-}t\ d)}/C_{min\ (4\text{-}t\ d)}$, where $C_{max(4\text{-}t\ d)}$ is the maximum plasma concentration from 4 to 78 days, and $C_{min\ (4\text{-}t\ d)}$ represents the minimum plasma concentration from 4 to 78 days. The smaller the $C_{max(4\text{-}t\ d)}/C_{min\ (4\text{-}t\ d)}$, the more constant the release of the depot. The $C_{max(4\text{-}t\ d)}/C_{min\ (4\text{-}t\ d)}$ values of Ris, 9-OH-Ris and the total active components were all significantly reduced in the Ris-m-SAIB depot, especially for the total active components, and the $C_{max(4\text{-}t\ d)}/C_{min\ (4\text{-}t\ d)}$ value was reduced from 40.7 ± 22.8 to 8.6 ± 4.5. This indicates that the drug release behavior of the Ris-m-SAIB depot was steadier than that of the Ris-SAIB depot.

### *In Vivo* Degradation of Ris-m-SAIB Depot

The *in vitro* drug release and *in vivo* pharmacokinetic studies showed that the Ris-m-SAIB depot exhibited a promising

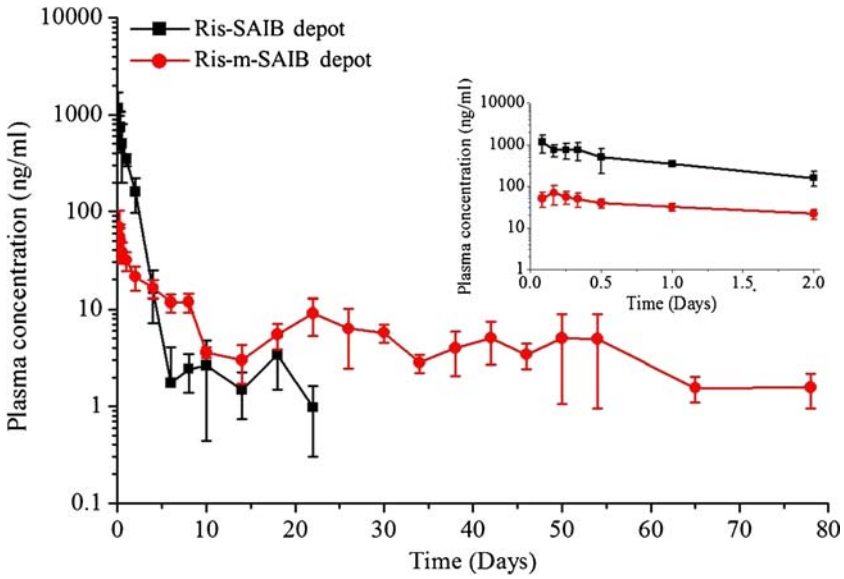


**Fig. 6** Plasma concentration-time profiles of active components (Ris plus 9-OH-Ris) after intramuscular injection of Ris-SAIB depot and Ris-m-SAIB depot (with a drug loading of 25 mg/g) to rats at a dose of 12.5 mg/kg (mean ± SD, $n = 6$).

**Table II** The Non-compartmental Model Pharmacokinetic Parameters of Ris After i.m. Administration of 25 mg/g Ris-SAIB Depot and Ris-m-SAIB Depot to Rats at a Dose of 12.5 mg/kg (mean ± S.D.; $n = 6$)

| Formulations | Ris-SAIB depot | Ris-m-SAIB depot |
|---|---|---|
| $AUC_{0\text{-}4d}$ (ng/mL·d) | 556.0 ± 142.0 | 66.7 ± 9.1 |
| $AUC_{4\text{-}t\ d}$ (ng/mL·d)[a] | 50.4 ± 8.9 | 225.3 ± 91.2 |
| $C_{max}$ (ng/mL) | 821.4 ± 347.3 | 40.8 ± 13.7 |
| $C_{s\ (4\text{-}t\ d)}$ | 2.8 ± 0.4 | 3.6 ± 0.4 |
| $C_{max}/C_{s(4\text{-}t\ d)}$ | 291.1 ± 94.4 | 11.3 ± 3.1 |
| $C_{max\ (4\text{-}t\ d)}$ | 7.8 ± 3.4 | 11.0 ± 0.2 |
| $C_{min\ (4\text{-}t\ d)}$ | 0.4 ± 0.4 | 1.1 ± 0.2 |
| $C_{max(4\text{-}t\ d)}/C_{min\ (4\text{-}t\ d)}$ | 32.4 ± 22.1 | 10.5 ± 2.7 |

[a] t was 22 days for Ris-SAIB depot, while t was 78 days for the Ris-m-SAIB depot

drug release behavior, as reflected by a low burst release followed by a steady and sustained release. In the literature, the degradation, metabolism and toxicity of SAIB have been extensively evaluated after oral administration to rats, dogs and humans (35–37). However, to the authors' knowledge, there has been little published data on the degradation of SAIB after intramuscular applications. In this study, the remaining depot mass at each predetermined time point was removed from the injection site, freeze-dried and weighed to assess the degradation of Ris-m-SAIB depot after intramuscular injection to rats.

As seen in Fig. 7, the depot showed little weight change during the first 4 days after administration, followed by a slow degradation after 4 days. After 78 days of injection, the mass remaining (%) was 38.64 ± 22.1%. The degradation kinetics fitted the first-order model well ($r = 0.9612$), with a fitted regression equation $\ln W_t = -0.0127 \times t + 4.517$, where $W_t$ represents the mass remaining (%) at any sampling time point (t), and t represents the sampling time point. The degradation

**Table III** The Non-compartmental Model Pharmacokinetic Parameters of 9-OH-Ris After i.m. Injection of 25 mg/g Ris-SAIB Depot and Ris/PLGA Microspheres Loaded SAIB Depots to Rats at a Dose of 12.5 mg/kg (mean ± S.D.; $n = 6$)

| Formulations | Ris SAIB depot (a) | Ris-m-SAIB depots (b) |
|---|---|---|
| $AUC_{0\text{-}4d}$ (ng/mL·d) | 440.1 ± 111.3 | 38.6 ± 16.1 |
| $AUC_{4\text{-}t\ d}$ (ng/mL·d)[a] | 28.4 ± 9.7 | 160.0 ± 50.4 |
| $C_{max}$ (ng/mL) | 536.9 ± 229.0 | 34.4 ± 16.9 |
| $C_{s\ (4\text{-}t\ d)}$ | 2.6 ± 1.7 | 2.5 ± 0.9 |
| $C_{max}/C_s$ | 339.2 ± 373.5 | 12.9 ± 3.1 |
| $C_{max\ (4\text{-}t\ d)}$ | 8.6 ± 4.9 | 7.6 ± 2.5 |
| $C_{min\ (4\text{-}t\ d)}$ | 0.12 ± 0.04 | 0.44 ± 0.22 |
| $C_{max(4\text{-}t\ d)}/C_{min\ (4\text{-}t\ d)}$ | 75.0 ± 44.8 | 19.7 ± 7.9 |

[a] t was 14 days for Ris-SAIB depot, while t was 78 days for the Ris-m-SAIB depot

**Table IV** The Non-compartmental Model Pharmacokinetic Parameters of the Active Components (Ris plus 9-OH-Ris) After i.m. Injection of 25 mg/g Ris-SAIB Depot and Ris-m-SAIB Depots to Rats at a Dose of 12.5 mg/kg (mean ± S.D.; $n = 6$)

| Formulations | Ris-SAIB depot (a) | Ris-m-SAIB depots (b) |
|---|---|---|
| $AUC_{0\text{-}4d}$ (ng/mL·d) | 996.1 ± 190.4 | 105.3 ± 24.4 |
| $AUC_{4\text{-}t\ d}$ (ng/mL·d)[a] | 78.8 ± 20.7 | 385.3 ± 126.6 |
| $AUC_{(0\text{-}\infty)}$ (ng/mL·d) | 1086.3 ± 207.1 | 534.4 ± 135.8 |
| $C_{max}$ (ng/mL) | 1283.2 ± 455.3 | 73.9 ± 30.6 |
| $C_{s\ (4\text{-}t\ d)}$ | 4.3 ± 1.0 | 7.5 ± 2.4 |
| $C_{max}/C_s$ | 311.4 ± 123.2 | 10.8 ± 4.8 |
| $C_{max\ (4\text{-}t\ d)}$ | 16.0 ± 8.7 | 16.9 ± 2.9 |
| $C_{min\ (4\text{-}t\ d)}$ | 0.6 ± 0.5 | 2.8 ± 2.2 |
| $C_{max(4\text{-}t\ d)}/C_{min\ (4\text{-}t\ d)}$ | 40.7 ± 22.8 | 8.6 ± 4.5 |

[a] t was 22 days for Ris-SAIB depot, while t was 78 days for the Ris-m-SAIB depot

half-life time ($t_{1/2}$) of Ris-m-SAIB depot can be calculated from the above equation ($t_{1/2} = 54.6$ days).

The results of the *in vivo* degradation study indicate that the Ris-m-SAIB depot was biodegradable after intramuscular injection to rats. As reported previously, the orally administered SAIB could be hydrolyzed by non-specific esterases to low molecular weight esters and other physiological components including glucose and fructose. The hydrolysates would be used in physiological processes and eventually metabolized to $CO_2$ (38). This might also be the case for the intramuscularly administered SAIB. In addition, histological examination of the muscle tissue adjacent to the depot on day 30 showed that the injected depot was surrounded by a large amount of inflammatory cells (Fig. 8). A number of small fragments of the depot, i.e., microspheres, were found dispersed in the inflammatory tissue. The surrounding inflammatory cells can phagocytize some molecules with a low molecular weight on the surface of the depot or particles with a size smaller than 10 μm (7, 33, 39, 40), which may accelerate the erosion and breakdown of the depot into small particles. The generated particles would be completely cleared via hydrolysis and phagocytosis. The detailed degradation mechanism after intramuscular application, however, still needs further investigation.

## Biocompatibility of the Ris-m-SAIB Depot

The *in vivo* biocompatibility of the Ris-m-SAIB depot was evaluated after intramuscular injection of approximately 0.1 g depot in rats. At 1, 2, 4, 6, 10, 14, 18, 22, 26, 30, 54 and 78 days, an incision was made at the injection site to expose the depot. Histological examination of the muscle tissue surrounding the depot was conducted. The representative images of the injection sites and histopathological images of the injection sites at 1, 10, 30 and 78 days are shown in Fig. 9.

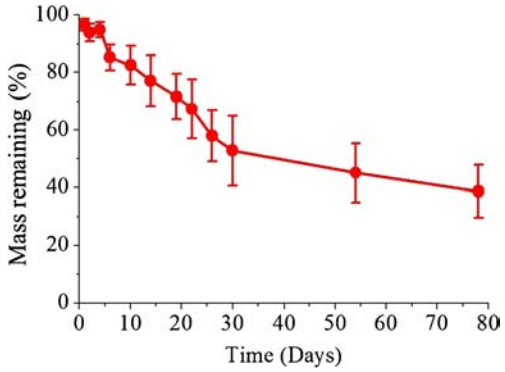


**Fig. 7** Mass remaining (%) *vs* time profiles of Ris-m-SAIB depot intramuscularly injected into rats at a dose of 12.5 mg/kg (equal to approximately 0.1 g depot) (mean ± SD; $n = 3$).

As shown in Fig. 9a–d, over the entire 78 day time period of this study, the injected depots were in close contact with muscle tissue, and did not cause any visible redness, swelling or purulent exudate at the site of injection.

On the first day after injection, the depot was surrounded by a transparent biomembrane (Fig. 9a). An obvious acute inflammatory response with the presence of neutrophils could be observed in the muscle tissue surrounding the depot (Fig. 9e). Furthermore, slight edema and hemorrhage (characterized by the presence of erythrocytes) accompanied the inflammation. This early acute inflammatory response may mainly be induced by the solvent diffusing from the depot and the mechanical injury created by injection.

After 10 days post-injection, visible blood capillaries were observed at the interface between the depot and the muscle tissue (Fig. 9b). Histopathological examination showed that a subacute inflammatory response, characterized by the infiltration of inflammation cells, including macrophages, lymphocytes and plasmocytes, was observed at the depot/tissue interface, as shown in Fig. 9f. Evident neovascularization was also observed in the muscle tissue surrounding the depot, which is in agreement with the result of Fig. 9b.

After 30 days post-injection, the biomembrane surrounding the depot became incrassated (Fig. 9c). The depot was divided into multi-units, being accompanied by the development of a fibrous capsule surrounding the depot. No inflammatory response in the adjacent muscle tissue was observed. Only a reduced inflammatory response was seen at the depot/tissue interface, as shown in Fig. 9g.

Finally, at 78 days post-injection, the volume of depot decreased significantly (Fig. 9d). The volume generated by the loss of depot was filled with fibroblasts (Fig. 9h). No inflammatory response was observed at the depot/tissue interface.

Over the entire 78-day time period of this study, the volume of depot decreased with time, demonstrating that the Ris-m-SAIB depot was biodegradable after intramuscular injection. Inflammation followed by neovascularization, fibrous encapsulation and fibrosis could be observed at the depot/tissue interface after the intramuscular injection of Ris-m-SAIB depot. The above tissue responses were similar to those caused by the polymers (PLA and PLGA) approved by the FDA (7, 41). This result suggests that the Ris-m-SAIB depot exhibited good biocompatibility with the muscle tissues.

## DISCUSSION

The *in vitro* drug release results indicate that the drug release profiles of the Ris-SAIB depot and Ris-m-SAIB depot both fitted the Higuchi model well. The kinetics of drug release from the hydrophobic SAIB depot can be described by the Higuchi Equation (42).

$$Q = A \cdot [D\varepsilon/k(2C_d - \varepsilon C_w)C_w t]^{1/2} \quad (1)$$

where, $Q$ represents the amount of drug release, $A$ represents the diffusion area, $D$ is the diffusion coefficient of drug, $\varepsilon$ is the porosity of the depot, $k$ is the tortuosity factor of the depot, $C_d$represents the concentration of dissolved drug in the depot, $C_w$ represents the drug solubility in the release medium and $t$ represents the time. As previously reported, the SAIB/EtOH systems could form a dense matrix structure after injection into the medium (25). The two SAIB depot systems (with and without drug-microspheres) in this study

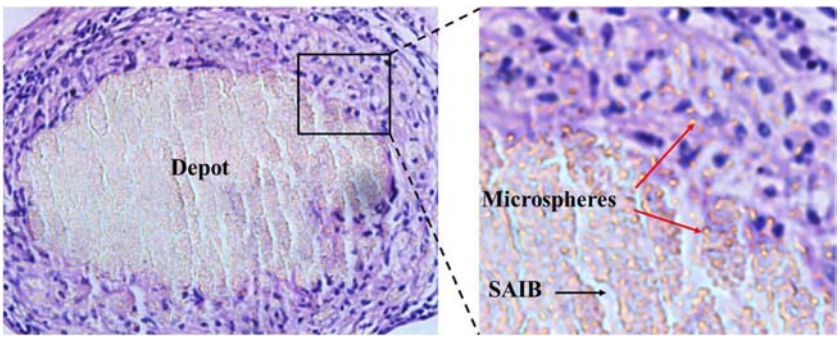


**Fig. 8** Representative pathological observations of injection sites after intramuscular administration of Ris-m-SAIB depot at 30 days. The magnification was x400.

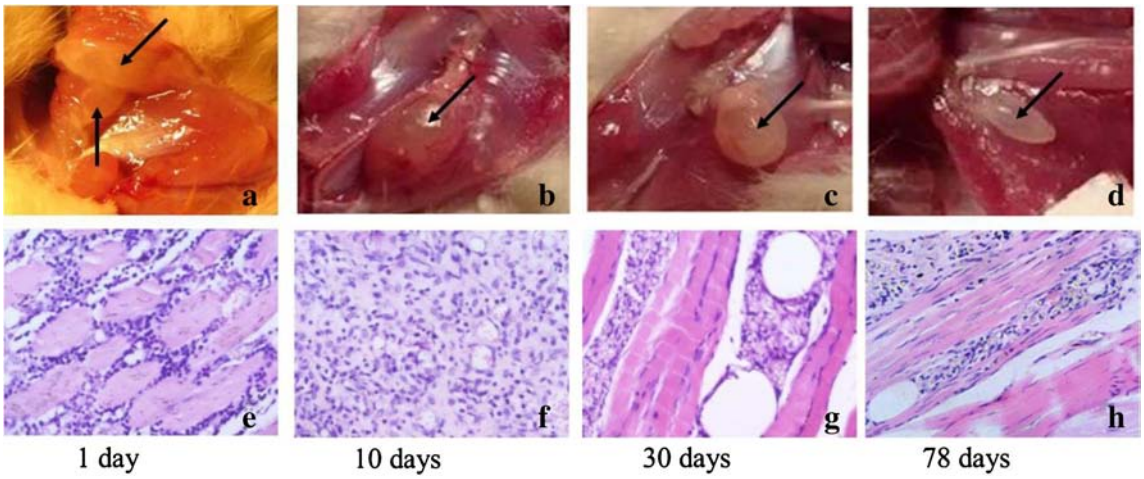


**Fig. 9** Representative *images* of the injection sites after administration of Ris-loaded microsphere-SAIB depot. Representative pathological observation of injection sites after intramuscular administration of Ris-loaded microsphere-SAIB *in situ* forming depot. The magnification was x400.

exhibited similar and constant porosity ($\varepsilon$) and a tortuosity factor ($k$). Eq. (1) can be written as

$$Q = A \cdot [D(2C_d - C_w)C_w t]^{1/2} \quad (2)$$

Due to the same depot matrix system, the diffusion area ($A$) and diffusion coefficient of drug ($D$) in Ris-m-SAIB depot were both similar to those of Ris-SAIB depot. The amount of drug release ($Q$) was mainly affected by the concentration of dissolved drug in the depot ($C_d$). For the Ris-SAIB depot, the concentration of dissolved drug ($C_{d1}$) was 18.01 mg/g. However, for the Ris-m-SAIB depot, most of the drug was encapsulated in the microspheres. The concentration of dissolved drug in the depot ($C_{d2}$) was only 0.52 mg/g, which was much lower than $C_{d1}$. On the first day after injection into the medium, the reduced $C_{d2}$ resulted in a significantly reduced burst release (only 0.64%), which was markedly lower than that of the Ris-SAIB depot (up to 12.16%).

After formation of the depot, as the SAIB was non-degradable *in vitro*, the diffusion area ($A$) and diffusion coefficient of drug in the SAIB depot ($D$) were both constant over the entire 60-day time period of the *in vitro* study. The concentration of dissolved drug in the depot ($C_d$) still played an important role in drug release. For the Ris-SAIB depot, the drug release rate was controlled by diffusion. For the Ris-m-SAIB depot, the drug should be released from the microspheres into

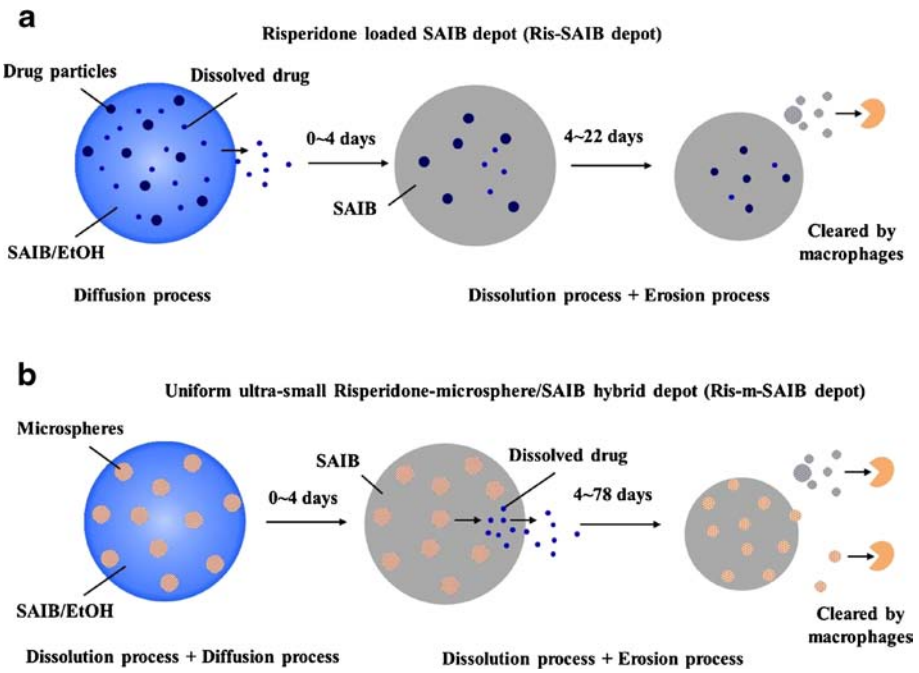


**Fig. 10** The *in vivo* drug release processes of (**a**) Ris-SAIB depot and (**b**) Ris-m-SAIB depot.

the SAIB depot first before it diffused into the medium. Therefore, the drug release rate was dissolution-controlled and then diffusion-controlled. As shown in Fig. 3 and Table I, the drug release rate of Ris-m-SAIB depot was slightly slower than that of Ris-SAIB depot from day 1 to day 8, whereas it was slightly more rapid than that of Ris-SAIB depot from day 10 to day 60. This indicates that the initial drug release rate of Ris-m-SAIB depot was controlled by the rate of drug dissolved and diffused from microspheres into the SAIB matrix, while the subsequent drug release rate was influenced by the residual drug in the depot.

After intramuscular injection into rats, the Ris-m-SAIB depot exhibited a much lower total active components plasma concentration level during the first 4 days and a steadier drug release profile from 4 to 78 days than the Ris-SAIB depot, which is in agreement with the results of the *in vitro* study. According to the *in vivo* pharmacokinetics and degradation study, the *in vivo* drug release processes of the two depot systems are illustrated in Fig. 10. In the first 4 days after administration, minimal degradation occurred to the Ris-m-SAIB depot, indicating that the degradation rate of depot was far lower than the drug diffusion rate. Therefore, the drug release rates, controlled by diffusion, can also be described by Eq. (2), $Q=A\cdot[D\ (2C_d\text{-}C_w)\ C_w t]^{1/2}$. In comparison with the *in vitro* study, *in vivo*, the depot can be dispersed into multi-units, resulting in a larger diffusion area ($A$), and so the dissolved drug in the depot was consequently released rapidly from the depot into body fluid, leading to a high total active components plasma concentration during the first 4 days. Due to the high $C_d$ (18.01 mg/g) of the Ris-SAIB depot, almost 91.7% ($AUC_{0-4\ d}/AUC_{(0\text{-}\infty)}$%) of drug was released into the body fluid during the first 4 days, while the $C_{max}$ of total active components was up to 1283.2±455.3 ng/ml. However, for the Ris-m-SAIB depot, the $C_d$ was only 0.52 mg/g. The drug release rate was limited by the dissolution of drug from microspheres into the depot. Only 19.7% ($AUC_{0-4\ d}/AUC_{(0\text{-}\infty)}$%) of the drug was released during the first 4 days. The $C_{max}$ of the total active components significantly decreased to 73.9± 30.6 ng/ml. Also, 4 days post-injection, most of the dissolved drug in the two depot systems had been released, leaving the undissolved drug particles or microspheres containing drug locked in the depot, and thus the drug dissolution rate into SAIB became the rate-limiting process for the subsequent drug release. In addition, the degradation of SAIB depot might also slightly accelerate the drug release. As only a small few amount of undissolved drug was locked in the depot, the Ris-SAIB depot exhibited a lower total drug concentration level (ranging from 0.4~7.8 ng/ml) from day 4 to day 22 and a shorter release period (22 days) than the Ris-m-SAIB depot (the drug concentration ranging from 1.55~ 16.30 ng/ml with a 78-days released period).

Based on the above results, the concentration of dissolved drug ($C_d$) in the depot was the main factor controlling the burst release. For depot systems with and without drug-loaded microspheres, the remaining drug dispersed in the depot after the initial release affected the long time period of the drug release profile whereby it can determine the sustained release period and the plasma concentration at the steady stage. Therefore, the incorporation of Ris-loaded microspheres into SAIB can not only effectively reduce the burst release of SAIB but it can also prolong the drug release time period, allowing the microsphere/SAIB hybrid depot to act as a long-term drug delivery system with a steady drug release rate.

## CONCLUSIONS

Compared with Ris-SAIB depot, the Ris-m-SAIB depot reduced the *in vitro* burst release from 12.16 to 0.64%. After intramuscular injection into rats, the $C_{max}$ of total active components was significantly reduced from 1283.2±455.3 to 73.9±30.6 ng/ml, while the amount of drug release over the first 4 days ($AUC_{4-t\ d}/AUC_{(0\text{-}\infty)}$%) was reduced from 91.7 to 19.7%. The $C_{max}/C_{s(4\text{-}t\ d)}$, representing the burst release *in vivo*, was significantly reduced from 311.4±123.2 to 10.8±4.8, approximately a 30-fold reduction. The drug release time period was prolonged from 22 to 78 days with a steady drug release rate reflected by the low $C_{max(4\text{-}t\ d)}/C_{min\ (4\text{-}t\ d)}$values of 8.6±4.5. By encapsulating Ris-microspheres in SAIB, the dissolved drug concentration ($C_d$) after suspension in SAIB depot was significantly reduced, resulting in a decreased burst release and a prolonged drug release period with a steady rate.

The Ris-m-SAIB depot is biodegradable after intramuscular administration to rats with a degradation half-life time ($t_{1/2}$) of 54.6 days. The degradation kinetics can be described by the first-order model. No tissue necrosis or abscess formation was observed in the muscle tissue adjacent to the depot. Inflammation, neovascularization, fibrous encapsulation and fibrosis were typical tissue reactions. The Ris-m-SAIB depot exhibit good biocompatibility with the muscle tissues.

The results of this study suggest that the uniform ultrasmall microsphere/SAIB hybrid depot is an excellent biodegradable sustained release injection drug delivery system with a low initial burst release, a continuous and steady drug release profile, and good biocompatibility.

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## REFERENCES


1. Uhrich KE, Cannizzaro SM, Langer RS, Shakesheff KM. Polymeric systems for controlled drug release. Chem Rev. 1999;99(11):3181–98.
2. Heres S, Lambert M, Vauth R. Treatment of early episode in patients with schizophrenia: the role of long acting antipsychotics. Eur Psychiatry. 2014;29 Suppl 2(14):1409–13.
3. Hoybye C, Christiansen JS. Growth hormone replacement in adults—current standards and new perspectives. Best Pract Res Clin Endocrinol Metab. 2015;29(1):115–23.
4. Jiang Y, Wang F, Xu H, Liu H, Meng Q, Liu W. Development of andrographolide loaded PLGA microspheres: optimization, characterization and in vitro-in vivo correlation. Int J Pharm. 2014;475(1–2):475–84.
5. Doan TV, Couet W, Olivier JC. Formulation and in vitro characterization of inhalable rifampicin-loaded PLGA microspheres for sustained lung delivery. Int J Pharm. 2011;414(1–2):112–7.
6. Hu Z, Liu Y, Yuan W, Wu F, Su J, Jin T. Effect of bases with different solubility on the release behavior of risperidone loaded PLGA microspheres. Colloids Surf B: Biointerfaces. 2011;86(1):206–11.
7. Shive MS, Anderson JM. Biodegradation and biocompatibility of PLA and PLGA microspheres. Adv Drug Deliv Rev. 1997;28(1):5–24.
8. Schwendeman SP, Shah RB, Bailey BA, Schwendeman AS. Injectable controlled release depots for large molecules. J Control Release. 2014;190:240–53.
9. Wischke C, Schwendeman SP. Principles of encapsulating hydrophobic drugs in PLA/PLGA microparticles. Int J Pharm. 2008;364(2):298–327.
10. Wang J, Wang BA, Schwendeman SP. Characterization of the initial burst release of a model peptide from poly(D, L-lactide-co-glycolide) microspheres. J Control Release. 2002;82(2–3):289–307.
11. Yang J, Wilson M, Celniker A. Recombinant human growth hormone poly(lactic-co-glycolic acid) (PLGA) microspheres provide a long lasting effect. J Control Release. 1997;49(97):193–205(13).
12. Ereshefsky L, Mannaert E. Pharmacokinetic profile and clinical efficacy of long-acting risperidone: potential benefits of combining an atypical antipsychotic and a new delivery system. Drugs R&D. 2005;6(3):129–37.
13. Eerdekens M, Van Hove I, Remmerie B, Mannaert E. Pharmacokinetics and tolerability of long-acting risperidone in schizophrenia. Schizophr Res. 2004;70(1):91–100.
14. Sun F, Sui C, Teng L, Liu X, Teng L, Meng Q, *et al.* Studies on the preparation, characterization and pharmacological evaluation of tolterodine PLGA microspheres. Int J Pharm. 2010;397(1–2):44–9.
15. Luan X, Bodmeier R. Modification of the tri-phasic drug release pattern of leuprolide acetate-loaded poly(lactide-co-glycolide) microparticles. Eur J Pharm Biopharm. 2006;63(2):205–14.
16. Su Z, Sun F, Shi Y, Jiang C, Meng Q, Teng L, *et al.* Effects of formulation parameters on encapsulation efficiency and release behavior of risperidone poly(D, L-lactide-co-glycolide) microsphere. Chem Pharm Bull (Tokyo). 2009;57(11):1251–6.
17. Husmann M, Schenderlein S, Luck M, Lindner H, Kleinebudde P. Polymer erosion in PLGA microparticles produced by phase separation method. Int J Pharm. 2002;242(1–2):277–80.
18. Berkland C, King M, Cox A, Kim K, Pack DW. Precise control of PLG microsphere size provides enhanced control of drug release rate. J Control Release. 2002;82(1):137–47.
19. Berkland C, Kim K, Pack DW. PLG microsphere size controls drug release rate through several competing factors. Pharm Res. 2003;20(7):1055–62.
20. Gao Z, Ding P, Zhang L, Shi J, Yuan S, Wei J, *et al.* Study of a pingyangmycin delivery system: Zein/Zein-SAIB in situ gels. Int J Pharm. 2007;328(1):57–64.
21. Kraeling RR, Barb CR, Rampacek GB, Thompson Jr DL, Gibson JW, Sullivan SA, *et al.* Luteinizing hormone response to controlled-release deslorelin in estradiol benzoate primed ovariectomized gilts. Theriogenology. 2000;53(9):1681–9.
22. Shi X, Lin X, Yao C, Shen L, Feng Y. Injectable long-acting in situ forming systems for Radix Ophiopogonis polysaccharide. Int J Biol Macromol. 2015;72:553–9.
23. Okumu FW, Dao LN, Fielder PJ, Dybdal N, Brooks D, Sane S, *et al.* Sustained delivery of human growth hormone from a novel gel system: SABER. Biomaterials. 2002;23(22):4353–8.
24. Pechenov S, Shenoy B, Yang MX, Basu SK, Margolin AL. Injectable controlled release formulations incorporating protein crystals. J Control Release : Off J Control Release Soc. 2004;96(1):149–58.
25. Lin X, Yang S, Gou J, Zhao M, Zhang Y, Qi N, *et al.* A novel risperidone-loaded SAIB-PLGA mixture matrix depot with a reduced burst release: effects of solvents and PLGA on drug release behaviors in vitro/in vivo. J Mater Sci Mater Med. 2012;23(2):443–55.
26. Lu Y, Tang X, Cui Y, Zhang Y, Qin F, Lu X. In vivo evaluation of risperidone-SAIB in situ system as a sustained release delivery system in rats. Eur J Pharm Biopharm. 2008;68(2):422–9.
27. Huang X, Brazel CS. On the importance and mechanisms of burst release in matrix-controlled drug delivery systems. J Control Release. 2001;73(2–3):121–36.
28. Lu YX, Yu YL, Tang X. Sucrose acetate isobutyrate as an in situ forming system for sustained risperidone release. J Pharm Sci. 2007;96(12):3252–62.
29. Hogan M. DURECT Receives Complete Response Letter from FDA for POSIDUR™ (SABER®-Bupivacaine). 2014 February 12. Available from: http://phx.corporate-ir.net/phoenix.zhtml?c=121590&p=irol-newsArticle&ID=1899590.
30. Qi F, Wu J, Yang T, Ma G, Su Z. Mechanistic studies for monodisperse exenatide-loaded PLGA microspheres prepared by different methods based on SPG membrane emulsification. Acta Biomater. 2014;10(10):4247–56.
31. Qi F, Wu J, Fan QZ, He F, Tian GF, Yang TY, *et al.* Preparation of uniform-sized exenatide-loaded PLGA microspheres as long-effective release system with high encapsulation efficiency and bio-stability. Colloids Surf B: Biointerfaces. 2013;112(3):492–8.
32. Piacentini E, Drioli E, Giorno L. Membrane emulsification technology: twenty-five years of inventions and research through patent survey. J Membr Sci. 2014;468(20):410–22.
33. Thomas C, Gupta V, Ahsan F. Particle size influences the immune response produced by hepatitis B vaccine formulated in inhalable particles. Pharm Res. 2010;27(5):905–19.
34. Tran VT, Benoit JP, Venier-Julienne MC. Why and how to prepare biodegradable, monodispersed, polymeric microparticles in the field of pharmacy? Int J Pharm. 2011;407(1–2):1–11.
35. Procter BG, Chappel CI. Subchronic toxicity studies of sucrose acetate isobutyrate (SAIB) in the rat and dog. Food Chem Toxicol. 1998;36(2):101–10.
36. Reynolds RC. Metabolism and pharmacokinetics of sucrose acetate isobutyrate (SAIB) and sucrose octaisobutyrate (SOIB) in rats, dogs, monkeys or humans: a review. Food Chem Toxicol. 1998;36(2):95–9.
37. Reynolds RC, Chappel CI. Sucrose acetate isobutyrate (SAIB): historical aspects of its use in beverages and a review of toxicity studies prior to 1988. Food Chem Toxicol. 1998;36(2):81–93.

38. Phillips JC, Kingsnorth J, Rowland I, Gangolli SD, Lloyd AG. Studies on the metabolism of sucrose acetate isobutyrate in the rat and in man. Food Cosmet Toxicol. 1976;14(5):375–80.
39. Gogolewski S, Jovanovic M, Perren SM, Dillon JG, Hughes MK. Tissue response and in vivo degradation of selected polyhydroxyacids: polylactides (PLA), poly(3-hydroxybutyrate) (PHB), and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHB/VA). J Biomed Mater Res. 1993;27(9):1135–48.
40. Huffman K, Casey D. Effect of carboxyl end groups on hydrolysis of polyglycolic acid. J Polym Sci Polym Chem Ed. 1985;23(7):1939–54.
41. Kazazi-Hyseni F, Zandstra J, Popa ER, Goldschmeding R, Lathuile AA, Veldhuis GJ, *et al.* Biocompatibility of poly(d, l-lactic-co-hydroxymethyl glycolic acid) microspheres after subcutaneous and subcapsular renal injection. Int J Pharm. 2015;482(1–2):99–109.
42. Pajander J, Soikkeli AM, Korhonen O, Forbes RT, Ketolainen J. Drug release phenomena within a hydrophobic starch acetate matrix: FTIR mapping of tablets after in vitro dissolution testing. J Pharm Sci. 2008;97(8):3367–78.