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Original Article

# Pharmacokinetics and pharmacodynamics of SCT800, a new recombinant FVIII, in hemophilia A mice

Ruo-lan GU[1, #], Liang LIU[1, #], Liang-zhi XIE[2], Wen-lin GAI[2], Si-shuo CAO[2], Zhi-yun MENG[1], Hui GAN[1], Zhuo-na WU[1], Jian LI[1], Ying ZHENG[1], Xiao-xia ZHU[1, *], Gui-fang DOU[1, *]

[1]*State Key Laboratory of Drug Metabolism, Hematological Pharmacology, Beijing Institute of Transfusion Medicine, Beijing 100850, China;* [2]*Cell Culture R&D Center, Peking Union Medical College & Chinese Academy of Medical Sciences, Beijing 100005, China*

**Aim:** SCT800 is a new third-generation recombinant FVIII agent that is undergoing promising preclinical study. This study aimed to investigate the pharmacokinetic and pharmacodynamic profiles of SCT800 in hemophilia A mice.
**Methods:** After hemophilia A mice were intravenously injected with single dose of SCT800 (80, 180, and 280 IU/kg) or the commercially available product Xyntha (280 IU/kg), pharmacokinetics profiles were evaluated based on measuring plasma FVIII: C. For pharmacodynamics study, dose-response curves of SCT800 and Xyntha (1–200 IU/kg) were constructed using a tail bleeding model monitoring both bleeding time and blood loss.
**Results:** Pharmacokinetics profile analysis showed a dose independency of SCT800 ranging from 80 to 280 IU/kg and comparable pharmacokinetic profiles between SCT800 and Xyntha at the doses tested. Pharmacodynamics study revealed comparable $ED_{50}$ values of SCT800 and Xyntha in the tail bleeding model: 14.78 and 15.81 IU/kg for bleeding time, respectively; 13.50 and 13.58 IU/kg for blood loss, respectively. Moreover, at the doses tested, the accompanying dose-related safety evaluation in the tail bleeding model showed lower hypercoagulable tendency and wider dosage range potential for SCT800 than Xyntha.
**Conclusion:** In hemophilia A mice, SCT800 shows comparable pharmacokinetics and pharmacodynamics to Xyntha at the doses tested, and possibly with better safety properties.

**Keywords:** hemophilia A; SCT800; Xyntha; third-generation recombinant FVIII; B domain-deleted rFVIII; pharmacokinetics; pharmacodynamics; hemophilia A mice; tail bleeding model

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## Introduction

Replacement therapy by prophylactic treatment using FVIII products is the first line of clinical therapy for hemophilia A[1-4]. Recombinant FVIII (rFVIII), which is not restricted by the fluctuating blood supply and contains the same hemostatic potency as plasma-derived FVIII[5-7], is increasingly becoming the alternative to plasma-derived FVIII. Research to eliminate the risk of infectious disease has resulted in the development of the newest third-generation of rFVIII[8-11]. However, manufacturing rFVIII was extremely difficult and its expression was two or three orders of magnitude lower than other recombinant proteins produced with similar strategies[11-13]. Very few third-generation rFVIII products are currently available (Advate, Xyntha/Refacto AF and Novoeight), thus resulting in high prices and shortages in the rFVIII supply worldwide[14-16]. The development of new and reliable rFVIII products is urgent for the hemophilic community.

SCT800 is a recent third-generation rFVIII agent that belongs to the B domain-deleted rFVIII (BDD-rFVIII) family according to its chemical structure. This molecule is produced in Chinese hamster ovary (CHO) cells without the addition of any human or animal components during production and formulation. Previous pharmaceutical studies confirmed satisfactory yields of SCT800, and *in vitro* studies demonstrated that SCT800 was fully functional with satisfactory efficacy and potency in many assays that measured FVIII activity.

In the current preclinical studies, we used a hemophilia A mouse model, which was derived from C57BL/6 mice by

[#] These authors contributed equally to this work.
[*] To whom correspondence should be addressed.
E-mail douguifang@vip.sina.com (Gui-fang DOU);
13681022512@163.com (Xiao-xia ZHU)
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genetically engineering an FVIII exon 16 knock-out[17, 18]. This hemophilia A mouse contained extremely low background levels of FVIII: C and following a trauma, bled significantly more than normal control mice. Furthermore, the hemophilia A mouse model was well accepted for the preclinical study of new rFVIII agents, as many studies performed with this model demonstrated good correlation with the subsequent clinical data[19, 20]. Thus, the hemophilia A mice were chosen for testing the pharmacokinetics and pharmacodynamics of SCT800. Naturally, the C57BL/6 mice containing normal FVIII: C levels, and from which the hemophilia A mouse was derived, was selected as the normal control in the current studies.

Additionally, a chromogenic substrate assay was selected for FVIII: C quantification in the current studies because both SCT800 and Xyntha belong to the BDD-rFVIII protein family[21, 22], which has discrepancies with the commonly used one-stage clotting method[23, 24]. Furthermore, the chromogenic substrate assay is now the European Pharmacopoeia reference method for FVIII concentrates because it provides robust assay conditions and high resolution and lacks negative interference from pre-activated FVIII molecules[25, 26].

The animal studies herein evaluated the pharmacokinetics and pharmacodynamics of SCT800, which was compared with an already marketed BDD-rFVIII reference product, Xyntha. The pharmacokinetics of SCT800 was evaluated by single iv administration of 80, 180 and 280 IU/kg, respectively, to measure the plasma FVIII: C activities. Pharmacokinetic parameters were based on non-compartmental methods. Pharmacodynamic properties were evaluated in a tail bleed model in hemophilia A mice, evaluating both bleeding time and blood loss.

## Materials and methods

### Chemicals and reagents

Xyntha was purchased from Wyeth (Lot: F97049, 250 IU/bottle, USA). SCT800 was provided by the Cell Culture R&D Center of Peking Union Medical College & Chinese Academy of Medical Sciences (Lot: 20130401A, 250 IU/bottle, China). BIOPHEN FVIII: C assay kits were obtained from HYPHEN BioMed (Lot: 11701-PK: 3, France). FVIII-deficient plasma was purchased from SIEMENS (Lot: 546562, Germany). The plasma-derived FVIII standard was purchased from the National Institutes for Food and Drug Control (Beijing, China). All other chemicals and reagents were of analytical grade and obtained from Sigma-Aldrich Inc unless otherwise noted.

### Mice

All procedures for the studies on experimental animals in this article were performed in accordance with the National Research Council's "Guide for the Care and Use of Laboratory Animals". Eight C57BL/6 mice used as the normal control (SPF grade, both sexes, 16–19 g) were obtained from the experimental animal center of the Academy of Military Medical Sciences, and 144 hemophilia A mice (SPF grade, both sexes, 18–32 g) were purchased from the Shanghai Biomodel Organism Science & Technology Development Co, Ltd (Shanghai, China), which passed the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) certification. The animals were kept in a barrier facility, group-housed and acclimatized for approximately two weeks. Room temperature was maintained at 20–22 °C, the humidity was 40%–70% and a 12 h light/12 h dark cycle was controlled automatically. A pelleted standard rodent diet was offered as well as fresh water from a local domestic supply.

### Pharmacokinetic studies

A total of 40 hemophilia A mice (both sexes, 18–32 g) were randomly assigned to three SCT800 groups (280, 180 and 80 IU/kg) and one Xyntha group (280 IU/kg) ($n$=10 per group, 5 male and 5 female). The SCT800 injection solutions were prepared at different concentrations, 16, 36 and 56 IU/mL, for use in administration to the 80, 180 and 280 IU/kg groups, respectively, so that there was a similar injection volume for the different dose groups. These dilutions were prepared with dilution buffer containing 0.25 mg/mL $CaCl_2$, 1.5 mg/mL histidine, 4.2 mg/mL arginine, 9 mg/mL NaCl, 0.1 mg/mL Tween 80 and 3 mg/mL saccharose (pH 7.0). The Xyntha injection solution was prepared at 56 IU/mL using a dilution buffer containing 0.25 mg/mL $CaCl_2$, 3 mg/mL saccharose, 1.5 mg/mL histidine, 18 mg/mL NaCl and 0.1 mg/mL Tween 80 (pH 7.0).

The hemophilia A mice from the 80, 180 and 280 IU/kg SCT800 groups were administered a single iv bolus injection of the 16, 36 and 56 IU/mL SCT800 injection solutions (5 mL/kg), respectively, in the tail vein, while the hemophilia A mice from the 280 IU/kg Xyntha group were administered the aforementioned 56 IU/mL Xyntha injection solution (5 mL/kg). Blood samples (approximately 25 μL) were micro-collected from the orbital plexus and anticoagulated in a 0.13 mol/L trisodium-citrate solution (9:1, $v/v$) at 0, 0.083, 1, 2, 4, 8, 12, 16, 24, 32 and 40 h post-administration. Then, the samples were immediately stabilized by adding a buffer (1:5, $v/v$) containing 50 mmol/L Tris, 9 mg/mL NaCl and 1.0% BSA (pH 7.5). After centrifugation at 4000×$g$ for 5 min, the supernatant plasma was collected and stored at -80 °C until FVIII: C analysis.

### Tail bleeding model studies

The tail bleeding model was used to evaluate the pharmacodynamics of SCT800 in hemophilia A mice. Six dose groups, 200, 100, 50, 20, 5 and 1.0 IU/kg, were distributed between the SCT800 and Xyntha reference groups ($n$=8 per group, 4 male and 4 female). Additionally, a negative control group of hemophilia A mice ($n$=8, hemophilia A mice, 4 male and 4 female) and a normal control group of C57BL/6 mice ($n$=8, C57BL/6 mice, 4 male and 4 female) were both set up in parallel to reflect the negative and normal level of FVIII: C activity.

The SCT800 and Xyntha injection solutions were both prepared and diluted to 40, 20, 10, 4, 1 and 0.2 IU/mL as described in "Pharmacokinetic studies" section and were used for administration of the 200, 100, 50, 20, 5 and 1.0 IU/kg dose groups, respectively, of SCT800 and Xyntha. The negative

and normal control groups were both administered with the vehicle blank dilution buffer.

Briefly, the mice were anesthetized by intraperitoneal administration of 2.0% pentobarbital sodium and placed on a heating pad to maintain body temperature. The tail was preheated for 10 min in 37 °C saline. The mice were administered 5 mL/kg of SCT800 injection solution, Xyntha injection solution, or vehicle blank dilution buffer via a catheter inserted in the jugular vein. Bleeding was initiated 5 min after dosing by cutting 4 mm off the tip of the tail with a scalpel, followed by submerging the tail in a tube with 10 mL of 37 °C saline. The bleeding time and blood loss were monitored for 30 min. Bleeding time was defined as the sum of the duration of all bleeding episodes in the 30-min observation period. Blood loss was determined by measuring the hemoglobin concentration collected in the saline (Cytoanalyze, MEK-7222K, Japan).

### FVIII: C analysis

FVIII: C was measured by a chromogenic substrate assay described by Lee *et al*[27, 28]. The FVIII: C concentration of SCT800 or Xyntha in the plasma was calculated from calibration curves prepared with the plasma-derived FVIII standard by the BIOPHEN FVIII: C assay kit following manufacturer's instructions. The absorbance was measured with a microplate reader (BIO-TEK, ELX800, USA) at 405 nm.

### Background prescreen of hemophilia A mice

All hemophilia A mice used in both the pharmacokinetic and pharmacodynamic studies were prescreened for their background FVIII: C activity using the aforementioned FVIII: C analysis method, thus ensuring that all hemophilia A mice were genetically deficient in FVIII.

### Data analysis and calculation

The calibration curve for FVIII: C determination was fitted with the four-parameter logistic regression model using Origin 7.5 software. The pharmacokinetic parameters including elimination rate constant ($k_e$), clearance (Cl), half-life ($t_{1/2}$), mean residence time (MRT), area under the concentration-time curve ($AUC_{last}$) and apparent volume of distribution ($V_d$) were all analyzed by Phoenix WinNonlin 6.0 software (Pharsight, Inc, USA) using a non-compartmental model. The maximum concentration ($C_{max}$) was also measured. The pharmacodynamic profile was evaluated using GraphPad Prism 5.0 (GraphPad Software, Inc, USA). The dose effect curve fitting was performed using a semilog sigmoidal dose response model. $ED_{50}$ was defined as the dose resulting in 50% of maximal activity, and it was calculated with the following equation: $Y=Bottom+(Top-Bottom)/(1+10^{(LogED_{50}-X)}\times HillSlope)$.

### Statistical analysis

The dose independency of SCT800 at different doses was analyzed by one-way analysis of variance (ANOVA). The pharmacokinetic parameters of SCT800 and Xyntha at 280 IU/kg were compared by *t*-test. In the tail bleeding study, the results for both bleeding time and blood loss were compared using *t*-test. The $ED_{50}$ of SCT800 and Xyntha calculated from either bleeding time or blood loss was statistically indicated with 95% Confidence Intervals (95% CI) values and compared using *F*-test. The FVIII: C value and the pharmacokinetic data were represented as the mean±SD, and the pharmacodynamic data were shown as the mean± SEM. All tests were two-sided, and a *P*-value of 0.05 or less was considered significant.

## Results

### Pharmacokinetics

The plasma FVIII: C levels of SCT800 in hemophilia A mice were measured by chromogenic substrate analysis after single iv administrations at 80, 180 and 280 IU/kg, respectively (Figure 1A). The pharmacokinetic parameters were calculated using the Phoenix WinNonlin 6.0 software with non-compartmental models. Figure 2 displayed the statistical data for $t_{1/2}$ (Figure 2A), $k_e$ (Figure 2B) and $V_d$ (Figure 2C) by ANOVA analysis, and for all these parameters, there were no significant differences within different dose groups. Figure 2D indicated a positive linear correlation between dose and $AUC_{last}$, as well as dose and $C_{max}$, with correlation coefficient square ($r^2$) values of 0.997 and 0.993, respectively, indicating a dose independency and linear kinetics profile of SCT800 in hemophilia A mice at doses ranging from 80 to 280 IU/kg.

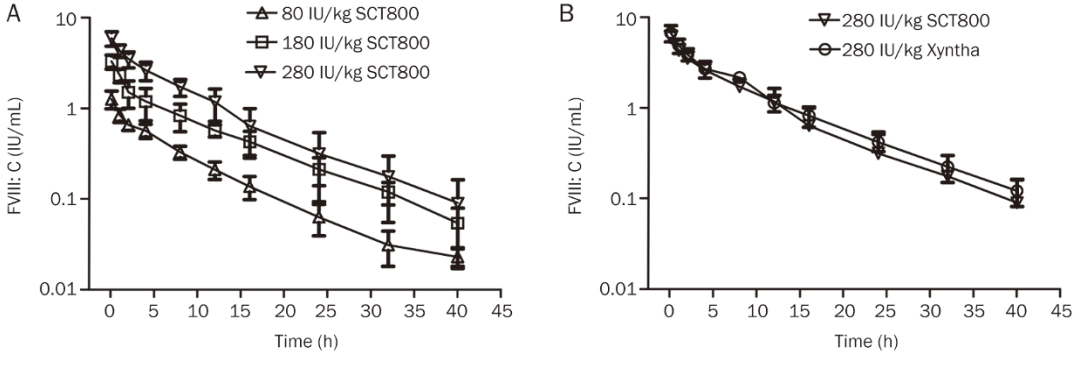


**Figure 1.** Mean plasma FVIII: C concentration-time curves of SCT800 and Xyntha in hemophilia A mice after single iv administration. (A) SCT800 of 80, 180 and 280 IU/kg, respectively; (B) SCT800 and Xyntha of 280 IU/kg, respectively. Data were shown as the mean±SD. *n*=10.

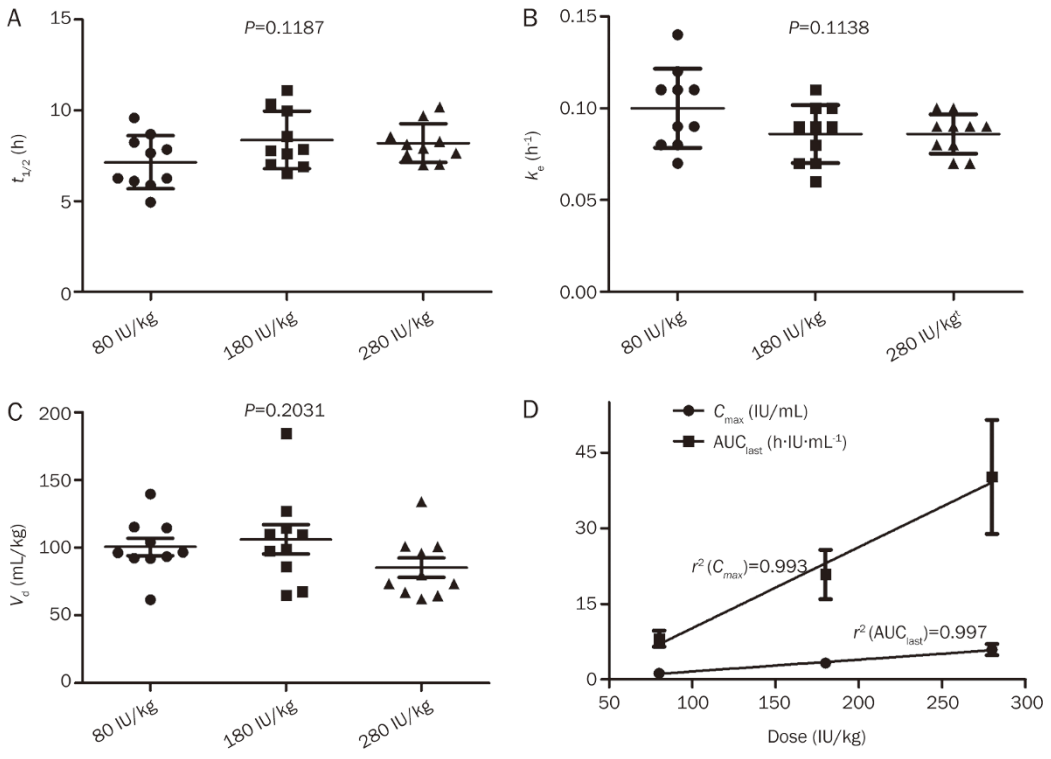


**Figure 2.** Pharmacokinetic dose independency analysis of SCT800 in hemophilia A mice after single iv administration at different doses. (A) ANOVA statistics of $t_{1/2}$; (B) ANOVA statistics of $k_e$; (C) ANOVA statistics of $V_d$; (D) linear correlation fitting between dose and $C_{max}$, as well as between dose and $AUC_{last}$, using the Origin 7.5 software. Data were shown as the mean±SD. $n$=10.

The pharmacokinetic comparison of SCT800 and Xyntha was conducted at 280 IU/kg by iv administration in hemophilia A mice. Figure 1B illustrated the mean plasma FVIII: C concentration-time curves of SCT800 and Xyntha. The calculated pharmacokinetic parameters of SCT800 and Xyntha, including $k_e$, $t_{1/2}$, $C_{max}$, $AUC_{last}$, $V_d$, Cl and MRT, were shown in Table 1 and compared using $t$-test. No significant differences were found in these parameters ($P$>0.05), indicating comparable pharmacokinetic profiles between SCT800 and Xyntha at the doses studied.

### Pharmacodynamics

Comparable dose response relationships of SCT800 and Xyntha in hemophilia A mice were found in the tail bleeding model at the doses studied. Therefore, the efficacies of these two products were both obviously correlated with dosage (Figure 3). There were no significant differences in the $ED_{50}$ values between SCT800 and Xyntha. Specifically, the $ED_{50}$ values calculated for bleeding time (Figure 3A) were 14.78 (95% CI: 6.720–32.52) and 15.81 IU/kg (95% CI: 0.4511–554.3), and the $ED_{50}$ values calculated for blood loss (Figure 3B) were 13.50 (95% CI: 5.101–35.71) and 13.58 (95% CI: 4.126–44.67) IU/kg, respectively, for SCT800 and Xyntha. Figure 4 further compared the hemostatic efficacy of SCT800 and Xyntha with hemophilia A mice as the negative control. These results indicated that both SCT800 and Xyntha at 1 IU/kg and 5 IU/kg demonstrated no obvious hemostatic efficacy ($P$>0.05 *vs* control hemophilia A mice) for both bleeding time (Figure 4A) and blood loss (Figure 4B). However, visible effects were achieved for both when the dose reached 20 IU/kg or higher,

**Table 1.** Pharmacokinetic parameters of haemophilia A mice after iv administration of 280 IU/kg SCT800 or Xyntha ($n$=10).

| Product | $k_e$ (h$^{-1}$) | $t_{1/2}$ (h) | $C_{max}$ (IU·mL$^{-1}$) | $AUC_{last}$ (h·IU·mL$^{-1}$) | $V_d$ (mL/kg) | Cl (mL·h$^{-1}$·kg$^{-1}$) | MRT (h) |
|---|---|---|---|---|---|---|---|
| SCT800 | 0.09±0.01 | 8.20±1.05 | 5.94±1.10 | 40.20±11.28 | 85.23±22.54 | 7.36±2.30 | 8.08±1.50 |
| Xyntha | 0.08±0.01 | 8.73±1.06 | 6.64±1.32 | 45.28±4.38 | 75.62±9.31 | 6.04±0.65 | 8.90±0.95 |
| *P*-value | 0.26 | 0.28 | 0.21 | 0.21 | 0.24 | 0.11 | 0.17 |

$k_e$, elimination rate constant; $t_{1/2}$, half life; $C_{max}$, maximal concentration; $AUC_{last}$, area under the concentration-time curve; $V_d$, apparent volume of distribution; Cl, clearance; MRT, mean residence time. Data were shown as mean±SD, compared using $t$-test.

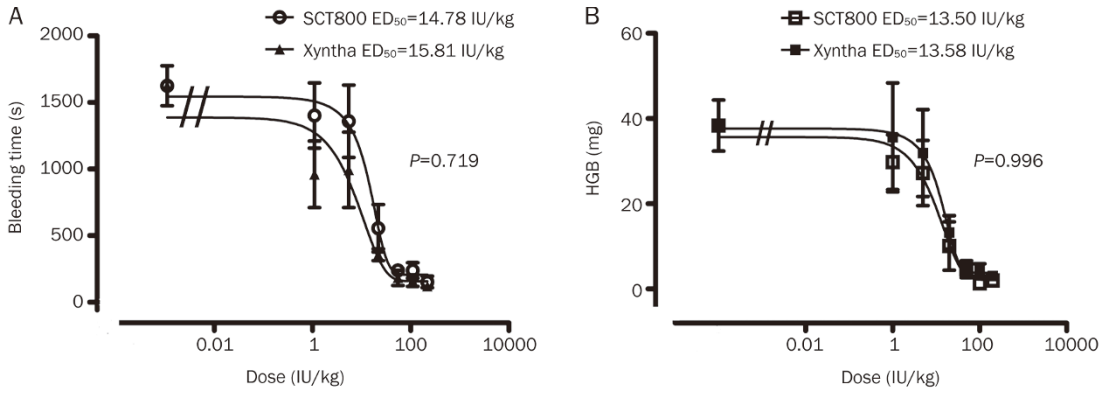


**Figure 3.** Sigmoidal dose response curve fits of bleeding time (A) and blood loss (B) in hemophilia A mice. SCT800 or Xyntha was iv administered 5 min before induction of bleeding by cutting a 4 mm tip off the tail. Data were shown as the mean±SEM ($n$=8). The $ED_{50}$ values were compared using $F$-test. The logistic regression equations follow. For bleeding time, SCT800: Y=219.6+(1434-219.6)/(1+10 ((Log14.78-X)×(-2.800))) and Xyntha: Y=182.6+(1002-182.6)/(1+10 ((Log 15.79-X)×(-5.664))), and for blood loss, SCT800: Y=1.820+(30.07-1.820)/(1+10^((Log13.50–X)×(-2.092))) and Xyntha: Y=3.138+(35.58-3.138)/(1+10^((Log 13.58- X)×(-2.129))).

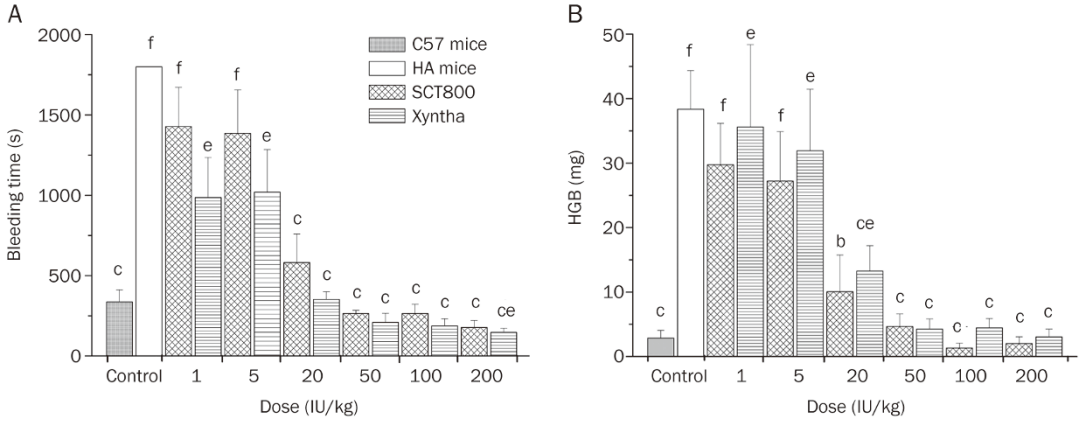


**Figure 4.** Statistical analysis of SCT800 and Xyntha in different dose groups for bleeding time (A) and blood loss (B) in hemophilia A mice. The results were shown as the mean±SEM ($n$=8). HA: hemophilia A mice, negative control; C57: C57BL/6 mice, normal control. Statistically significant differences compared with hemophilia A mice or C57BL/6 mice were indicated: $^{b}P<0.05$, $^{c}P<0.01$ *vs* HA mice; $^{e}P<0.05$, $^{f}P<0.01$ *vs* C57BL/6 mice.

which indicated similar hemostatic efficacy and potency of SCT800 and Xyntha in hemophilia A mice using the tail bleeding model at the doses studied.

Additionally, although the minimum doses of SCT800 and Xyntha to normalize bleeding time (defined as the minimum dose to shorten bleeding time to a similar level as the C57BL/6 normal control, as well as the minimum dose to take the $P$-value >0.05) were the same, 20 IU/kg (Figure 4A), the bleeding time of the high dose 200 IU/kg Xyntha group began to shorten, while the bleeding time of the 200 IU/kg SCT800 group was maintained at the normal level. Furthermore, the minimum doses of SCT800 or Xyntha to normalize blood loss (defined as the minimum dose to decrease blood loss to levels similar to the C57BL/6 normal control, as well as the minimum dose to make the $P$-value >0.05) were 20 and 50 IU/kg, respectively (Figure 4B). These results also indicated that SCT800 may contain lower hypercoagulable tendencies and a wider dosage range potential compared to Xyntha in hemophilia A mice at the doses studied.

## Discussion

The aims of the present study were to evaluate the *in vivo* pharmacokinetics and pharmacodynamics of a new BDD-rFVIII compound, SCT800, and to compare it with one of the most widely used commercially available BDD-rFVIII products, Xyntha. Taken together, our data demonstrated comparable pharmacokinetics and pharmacodynamics of SCT800 and Xyntha in hemophilia A mice.

There were two main findings in the present study. First, this was the first time a dose independency and linear kinetics profile of SCT800 were demonstrated in hemophilia A mice at doses ranging from 80 to 280 IU/kg. Moreover, the pharmacokinetic data obtained, such as $t_{1/2}$, was consistent with data from other similar rFVIII concentrates[19, 29, 30]. Second, comparable pharmacokinetic and pharmacodynamic profiles of SCT800 and Xyntha were first demonstrated in a hemophilia

A mice model. The pharmacodynamic data, especially the $ED_{50}$ value, was also consistent with data from studies of other rFVIII concentrates. Pan *et al*[31] reported an $ED_{50}$ of 21 IU/kg for rFVIII-FS, and Elm *et al*[19] reported an $ED_{50}$ of 28 IU/kg for Advate, both of which were very similar to our data.

Additionally, the accompanying dose-related safety evaluation using the tail bleeding model was also preliminarily analyzed and discussed by comparison with the C57BL/6 normal control mice. There were two factors taken together to analyze and compare the safety profiles of SCT800 and Xyntha. The first was the bleeding time for hypercoagulability evaluation because although bleeding time was not the most commonly used parameter for clinically judging hypercoagulability, it was yet another important indicator for screening bleeding or clotting disorders; thus, the shortened bleeding time *in vivo* always suggested a condition more susceptible to hypercoagulability risks[32, 33]. In the present study, the bleeding time of the hemophilia A mice treated with the 200 IU/kg dose of SCT800 still maintained a normal level, whereas the bleeding time for the same 200 IU/kg dose of Xyntha began to be lower than normal, indicating that SCT800 was less susceptible to hypercoagulability than Xyntha in hemophilia A mice at the doses studied. The other factor was the potential dosage range, which was evaluated for both the effective (the minimum dosage to normalize blood loss or bleeding time) and safe (the maximal dose to maintain normal for blood loss or bleeding time) doses. In the present study, the potential dosage range of SCT800 was relatively wider than Xyntha when evaluated for either blood loss or bleeding time. Based on these two factors, we suggested that SCT800 may contain lower hypercoagulable tendency and wider dosage range potential compared to Xyntha in hemophilia A mice at the doses studied.

There is, however, one limitation in the present study. As an accompanying safety analyses of the pharmacodynamic studies, the data available in this study were too limited to fully illustrate the safety profiles of SCT800. Further studies are needed and should be included in subsequent toxicity and safety evaluations. To emphasize the difference between bleeding times for SCT800 and Xyntha compared to the normal situation, one or two higher doses (such as 250 and 300 IU/kg) should be further studied in the tail bleeding model. Furthermore, parameters such as activated partial thromboplastin time and thromboelastography, which will contribute to further clarification of the hypercoagulable tendency *in vivo*[34] but were not achieved in these studies due to the limited amount of mouse blood, should be further investigated.

In conclusion, we have been the first to clarify the pharmacokinetics and pharmacodynamics of SCT800, a newly introduced third-generation rFVIII agent, and have compared it to a commercially available product, Xyntha, in hemophilia A mice. Pharmacokinetic data indicated a dose independency of SCT800 within the 80–280 IU/kg range, and comparable pharmacokinetic profiles between SCT800 and Xyntha at the doses studied. Pharmacodynamic data of comparable $ED_{50}$ values for SCT800 and Xyntha demonstrated comparable efficacy and potency of these two products, utilizing both bleeding time and blood loss in the hemophilia A mice tail bleeding model. In addition, SCT800 showed lower hypercoagulable tendency and wider dosage range potential compared to Xyntha in hemophilia A mice at the doses tested, which needs further evaluation and confirmation in subsequent toxicity and safety studies.

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## Author contribution

Ruo-lan GU, Xiao-xia ZHU, Liang-zhi XIE and Gui-fang DOU designed the research; Liang LIU, Ruo-lan GU, Wen-lin GAI, and Zhi-yun MENG analyzed the data; Ruo-lan GU and Liang LIU wrote the manuscript; and Liang LIU, Ruo-lan GU, Si-shuo CAO, Hui GAN, Zhuo-na WU, Jian LI, Ying ZHENG and Xiao-xia ZHU performed the experiments.

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