International Immunopharmacology 75 (2019) 105807

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

A randomized controlled dose-escalation study of SSS07, a humanized rabbit anti-human TNF alpha antibody, in healthy Chinese adults

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ARTICLE INFO

Keywords:
SSS07
Antibodies
TNF-α
PK
Immunogenicity

ABSTRACT

Aims: SSS07 is a rabbit derived humanized anti-human TNF-α antibody. This study aimed to explore the pharmacokinetics, safety, and immunogenicity of SSS07 when administrated subcutaneously in healthy adults.
Methods: This was a double-blind, dose-escalation study of SSS07 in 71 adults. Dose cohorts were set to 5 mg, 15 mg, 30 mg, 50 mg, 75 mg, and 100 mg. In each dosage group, other than 100 mg, twelve healthy participants were randomly assigned to receive a single dose of SSS07 (n = 10) or placebo (n = 2). Blood samples were taken for pharmacokinetics and immunogenicity analysis.
Results: No deaths, serious adverse events or drug-related withdrawals occurred in this trial. No drug limited toxicity appeared. After subcutaneous injection, SSS07 was absorbed slowly with T_{max} ranging from 60 to 264 h but eliminated quickly with a short half-life ranging from 21.69 to 78.4 h (1–3 days). From 5 mg to 100 mg, dose-exposure proportionality analysis found a 90% confidence interval (CI) of β of C_{max} (1.015–1.193), AUC_{0-t} (1.096–1.263) and $AUC_{0-\infty}$ (0.999–1.174) partially within the range 0.926–1.074. The plasma concentration of TNF-α decreased significantly post-dose, but a few days later, levels of TNF-α elevated rapidly and exceeded its baseline value. All participants receiving SSS07 were found to be anti-drug antibody positive during the study.
Conclusions: A single-dose injection of SSS07 was safe and well-tolerated in healthy adults. Doses of SSS07 from 5 mg to 100 mg could not be regarded as nonlinear, based on dose-exposure proportionality analysis. A high incidence of anti-drug antibodies indicated strong immunogenicity, which may influence the pharmacokinetics profile and interfere with the TNF-α inhibition capability of SSS07.

1. Introduction

Tumor necrosis factor alfa (TNF-α) was found at the apex of pro-inflammation factors exhibiting a dominant contribution to rheumatoid arthritis (RA) [1,2]. Therefore, neutralizing the amount of TNF-α has become a pivotal target to control inflammation in RA. Anti TNF-α monoclonal antibodies (mAbs) could bind to TNF-α directly and inhibit the downstream inflammation reaction [3]. So the application of anti-TNF-α monoclonal antibodies alone, or in combination with disease-modifying anti-rheumatic drugs or methotrexate, has achieved great success in RA therapy [4–6].

Advantages parallel drawbacks in the currently available TNF-α inhibitors. Etanercept, a human tumor necrosis factor receptor (TNFR) p75-Fc fusion protein, binds to TNF-α with high specificity, but the

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https://doi.org/10.1016/j.intimp.2019.105807
Received 30 May 2019; Received in revised form 3 August 2019; Accepted 4 August 2019
Available online 12 August 2019
1567-5769/

dimeric structure causes low combination rates [7]. Infliximab is a chimeric monoclonal antibody and binds to TNF-α with high affinity, but the mouse-derived sequences induce the expression of human anti-mouse antibody [1]. Adalimumab, the first fully human anti-TNF-α monoclonal antibody, shows low immunogenicity in patients with RA, and in recent years it has topped the list of the best-selling drugs on a global scale. However, the high cost restricts its prescription in RA patients.

It is reported that compared with antibodies from other sources, rabbit monoclonal antibodies have a wider repertoire, higher binding affinity, and are easier to be humanized. Moreover, humanized rabbit mAbs retain high affinity and specificity of the parental mAbs [8–10]. Nowadays, several rabbit derived mAbs are under investigation, and some are expected to transition from preclinical research to clinical trials [9]. SSS07, a highly humanized IgG1 antibody, is derived from rabbit mAbs with unique complementarity-determining regions. SSS07 could bind to TNF-α with high specificity and affinity. Compared with other anti-TNF-α antibodies on the market, SSS07 possesses the identical mechanism in TNF-α inhibition but has a different antigen-binding epitope. So SSS07 may still have therapeutic efficiency when immune tolerance against other TNF-α inhibitors occurs.

SSS07 in pre-clinical investigations was of high bio-availability and low immunogenicity. And in animal models, SSS07 displayed the same disease remission ability compared with adalimumab (Humira). In addition, no dose accumulation appeared with repeated SSS07 injections. Given that SSS07 was safe and well-tolerated in RA management in well-conducted pre-clinical studies, SSS07 was further investigated in healthy volunteers. It's noteworthy that this is the first clinical study that analyzed the immunogenicity of humanized rabbit anti-human antibody, rather than simply dealing with pharmacokinetics and demonstration of safety [9].

2. Materials and methods

2.1. Subjects

Healthy adults aged 18–45 years with a body mass index (BMI) ranging from 19.0 to 24.0 kg/m^2 were enrolled in this study. Subjects in each group had similar body weights, and the age range in each cohort was within ten years. Also, before the first dose of the study drug, subjects had to meet the following inclusion criteria: normal results for the laboratory safety test, electrocardiography (ECG), chest X-ray and other examination results within the screening period. Participants were excluded if they had taken any drugs in the two weeks preceding the screening date, or taken drugs harmful to major organs within three months; had a history of digestive system disease, cardiovascular disease or other clinically significant diseases; had HIV or hepatitis B or C; smoked or drank excessively; had acute/chronic infections. Pregnant and breastfeeding women were excluded.

2.2. Study drugs

SSS07 (batch number S201311001) and placebo for injection were prepared by Shen Yang Sunshine Bio-Pharmaceutical Co. Ltd. (China) as 50 mg prefilled syringes. Drugs were stored at 2–8 °C in the dark before administration.

2.3. Study design

This was a randomized, double-blind, placebo-controlled, single-dose ascending phase 1 study. The trial was conducted to investigate the pharmacokinetics, safety, and immunogenicity of SSS07 after abdominal subcutaneous injection in healthy Chinese adults. The study protocol was in accordance with the Declaration of Helsinki and approved by the ethics committee of Peking University People's Hospital (No. 2015PHA-008-01). The study was carried out by the phase 1 unit of Peking University People's Hospital. Before the initiation of the investigation, all subjects gave their informed consent.

This study planned to enroll 72 healthy participants in total and involved 6 ascending-dose cohorts. The doses were 5 mg, 15 mg, 30 mg, 50 mg, 75 mg, and 100 mg. In each cohort, there were 12 healthy subjects and 10 of them were randomly assigned to receive SSS07, while the other 2 participants received a placebo. The SSS07 higher-dosage research started only when safety was verified at the previous lower-dosage level.

This study consisted of a two-week screening period, and a subsequent one-day treatment period followed by three days of observation in hospital and follow-up visits ending on the 28th-day post-dose. If the subjects were anti-drug antibodies (ADA) positive on day 28, the duration of the study would be prolonged to six months to one year for immunogenicity evaluation. During the follow-up period, participants were regularly monitored by physical examination, laboratory tests, and ECG at each visit. Blood samples for pharmacokinetic assessments, TNF-α determination, and immunogenicity evaluation were collected at the scheduled time.

2.4. Methods

2.4.1. Safety assessments

The safety of single subcutaneous administration of SSS07 was evaluated in all participants involved. All subjects were monitored by physical examination, laboratory safety tests and electrocardiogram (ECG) after the randomization number generation. The severity of adverse events (AEs) were recorded by physicians as grade 1, grade 2, grade 3, grade 4, or grade 5, according to the Common Terminology Criteria for Adverse Events version 4.0 (CTCAE 4.0, 2009/9/15) published by the National Institute of Health and the National Cancer Institute (Bethesda, MD) [11]. A dose of 100 mg would be regarded as the maximum tolerated dose (MTD) if AEs were observed in < 30% of subjects in each cohort. Any dose under 100 mg with AE generation in > 30% of volunteers would be considered as MTD. Dose limiting toxicity (DLT) was verified when the severity of AE reached grade 3.

2.4.2. Pharmacokinetic assessments

Blood samples for pharmacokinetic assessments were obtained within 1 h preceding the injection, and at 4, 12, 24, 36, 48, 60, 72, 96, 120, 144, 168, 216, 264, 312, 360, 408, 456, 504, 576, and 672 h after dosing. Ten minutes of centrifugation was needed to separate the supernatant serum, and these serum samples were stored at −80 °C before analysis. Plasma levels of SSS07 were detected by enzyme-linked immunosorbent assay (ELISA) and the lower limit of quantification (LLOQ) was 15.625 ng/mL. Values determined lower than the LLOQ were set to zero. The pharmacokinetic parameters, the area under the plasma drug concentration-time curve (AUC), the time taken to reach the maximum concentration (T_{max}), peak concentrations of drug in serum (C_{max}), terminal elimination half-life ($t_{1/2z}$), apparent volume of distribution (V_d/F) and the apparent clearance (CL_z/F) were calculated using the plasma concentration-time data collected. The pharmacokinetic properties of SSS07 were assessed based on drug concentrations, and basic pharmacokinetic parameters calculated.

2.4.3. Immunogenicity assessments

Blood samples for ADA detection were collected during the screening period and on days 7, 15, 21 and 28 after drug administration. Subjects who were ADA positive on day 28 were further followed-up one year after the drug infusion, and serum specimens for antibody detection were obtained at month 3, 6, or 12. Validated bridging - ECLA was applied for ADA measurement, and the methodological sensitivity was 51.48 ng/mL. The threshold of the screening method and immunosuppression test method was the signal to noise ratio S/N = 1.08, and 13.4%, respectively.

2.4.4. Plasma TNF-α concentration assessments

Blood samples for TNF-α quantification were collected pre-dose, at hours 4, 72, 168, 264, 312, 408, 504, and 672 post-dose for TNF-α inhibition ability assessment. Blood samples were centrifuged and stored at −80 °C until analysis was conducted. Electrochemiluminescence assays (ECLA) were used to measure the plasma TNF-α concentrations, and the LLOQ was 0.078 pg/mL.

2.4.5. Statistical analysis

Statistical significance was set at $P < 0.05$ for all tests. Non-compartmental pharmacokinetics analysis for SSS07 was conducted using Phoenix® WinNonlin® 6.0.1 (Certara, Princeton, NJ, USA) and DAS (version 3.0). Demographic data sets and safety data were analyzed using SAS (Statistical Analysis System; SAS Institute Inc., Cary, NC, USA, version 9.2). Differences across treatment groups in PK parameters including C_{max}, AUC, $t_{1/2z}$, V_d /F, and CL_z/F were analyzed using analysis of variance (one-way ANOVA), while T_{max} was examined by Kruskal–Wallis H test. Meanwhile, all PK parameters except T_{max} were log-transformed in statistical analysis. A multiple comparisons test with Bonferroni correction was performed when necessary. Dose–exposure proportionality was assessed using a power model. The assumption was that the logarithm of the PK variable (C_{max} and AUC) is linearly related to the logarithm of dose: $\ln(PK) = \beta_0 + \beta_1 * \ln(dose)$. Dose proportionality was established when the 90% CI for the slope β_1 fell completely within the range 0.926–1.074 (the criterion interval: $1 + [\ln(0.80)/\ln(high/low)]$, $1 + [\ln(1.25)/\ln(high/low)]$) [12].

3. Results

3.1. Study subjects

A total of 71 healthy volunteers were enrolled in this study. In the 5 mg to 75 mg cohorts, 12 participants were randomly assigned to receive either the SSS07 (n = 10) or the placebo (n = 2) in each group. In the 100 mg group, due to the expiration date of study drugs, only 11 subjects received an injection (10 in SSS07, and 1 in the placebo group). All participants completed the trial without withdrawal. Participants were aged between 19 and 32 years, and the age range in each cohort did not exceed 10 years as planned. The detailed baseline characteristics of subjects are listed in Table 1.

3.2. Safety results

SSS07 was safe and well-tolerated when given subcutaneously with doses ranging from 5 mg to 100 mg. Adverse events (AEs) occurred in 42 volunteers (36 volunteers in the SSS07 group and 6 in the placebo group) but the severity of the AEs were all mild or moderate. The most frequent AE recorded was injection site reaction. No deaths or drug-related serious adverse events (SAEs) occurred. Therefore, the MTD of SSS07 was defined as 100 mg, and no DLT was observed. AEs that occurred frequently are listed in Supplementary Table 2.

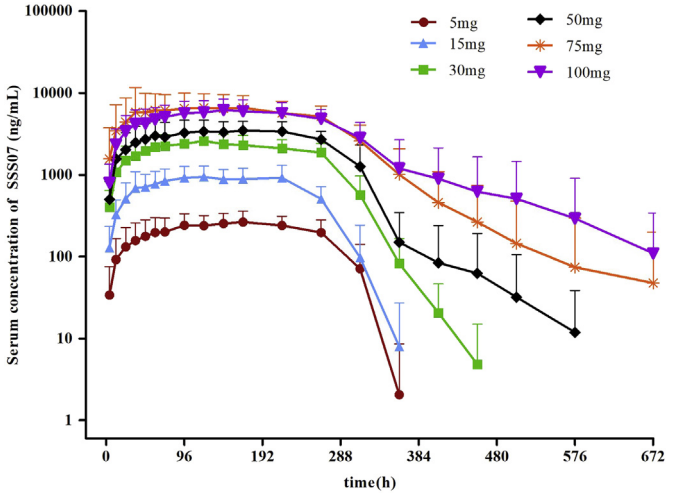

Fig. 1. Mean (SD) serum concentration-time profiles for SSS07 after a single dose subcutaneous administration in log-linear scale.

3.3. Pharmacokinetic results

The serum concentration-time profile of SSS07 is displayed in Fig. 1. SSS07 was slowly absorbed with the overall mean T_{max} of 148 h (60–264 h) for all dose groups, and serum SSS07 concentrations decreased rapidly 13 days after dosing with a short half-life ranging from 21.69 to 78.4 h. The estimated mean volume of distribution ranged from 1.78 to 6.76 L. $AUC_{0\text{-}t}$ ranged from 62.32 to 1874.83 h*ng/L. C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}\infty}$ of SSS07 increased with dose escalation. Results from ANOVA displayed significant differences in C_{max} ($P = 1.37 * 10^{-25}$), $AUC_{0\text{-}t}$ ($P = 5.38 * 10^{-28}$), $AUC_{0\text{-}\infty}$ ($P = 2.93 * 10^{-23}$), Vd/F ($P = 9.18 * 10^{-4}$) and $t_{1/2z}$ ($P = 0.012$), but no significant differences in CL_z/F ($P = 0.083$) among the 6 groups. Kruskal-Wallis H test didn't find any significant difference in T_{max} ($P = 0.370$). The multiple comparisons test showed that no significant differences existed in the comparison between the 30 mg and 50 mg groups in C_{max} ($P = 0.758$), $AUC_{0\text{-}t}$ ($P = 0.448$), and $AUC_{0\text{-}\infty}$ ($P = 0.168$). Moreover, C_{max} didn't show a significant difference in the comparison between 50 mg and 100 mg ($P = 0.099$). In the comparison between the 75 mg and 100 mg dose groups, no significant differences appeared in C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}\infty}$. Significant differences were found in all other dose group comparisons regarding C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}\infty}$. Significant difference also existed in Vd/F ($P = 0.001$) and $t_{1/2z}$ ($P = 0.005$) between the 5 mg and 30 mg dose cohorts. Vd/F in the 5 mg group was significantly larger than in the 50 mg ($P = 0.035$) and 75 mg ($P = 0.004$) groups. No significant differences were found in other comparisons regarding Vd/F and $t_{1/2z}$. The dose-proportionality analysis found that values of β of C_{max} (90% CI: 1.015–1.193), $AUC_{(0\text{-}t)}$ (90% CI: 1.096–1.263) and $AUC_{0\text{-}\infty}$ (90% CI: 0.999–1.174) were partially within the standard range 0.926–1.074. The detailed pharmacokinetic parameters are shown in Table 2.

Table 1
Baseline characteristics of subjects enrolled in study group. Mean (SD).

	5 mg	15 mg	30 mg	50 mg	75 mg	100 mg
Subjects (n)	10	10	10	10	10	10
Female/male (n)	6/4	4/6	3/7	4/6	4/6	5/5
Age	27.7 (2.9)	26.0 (3.4)	24.7 (3.3)	23.3 (2.4)	24.7 (1.5)	24.0 (2.8)
Weight (kg)	64.0 (9.4)	57.9 (6.7)	64.0 (5.6)	60.8 (6.1)	60.7 (4.8)	59.8 (7.2)
BMI[a] (kg/m^2)	21.9 (1.3)	21.2 (0.5)	22.0 (1.1)	21.1 (1.1)	21.4 (1.6)	21.4 (1.8)
TNF-α (pg/mL)	2.2 (0.58)	2.25 (0.73)	2.26 (0.34)	2.50 (0.46)	2.32 (0.31)	2.47 (0.33)

n number.
SD standard deviation, BMI body mass index.
[a] Body mass index was defined as weight/height in $meters^2$.

Table 2
Pharmacokinetics parameters of SSS07 administered in different doses.

PK parameters*	5 mg	15 mg	30 mg	50 mg	75 mg	100 mg
$t_{1/2z}$ (h)	78.40 (55.32)	36.9 (17.76)	21.69 (7.15)	31.45 (23.61)	34.78 (31.79)	38.74 (27.90)
T_{max} # (h)	60–216	96–264	96–216	60–264	60–168	72–216
C_{max} (μg/mL)	0.28 (0.10)	1.08 (0.37)	2.70 (0.85)	3.88 (1.24)	8.01 (5.30)	6.51 (2.28)
$AUC_{(0-t)}$ (h*μg/mL)	62.32 (21.84)	217.31 (75.58)	625.24 (189.67)	924.90 (313.38)	1874.83 (850.10)	1792.54 (679.00)
$AUC_{(0-inf)}$ (h*μg/mL)	80.52 (24.49)	217.25 (67.18)	626.41 (189.20)	951.20 (335.53)	1884.51 (848.39)	1806.69 (690.32)
Vd/F (L)	6.79 (4.14)	4.20 (2.73)	1.78 (1.23)	2.52 (1.80)	2.23 (2.01)	3.34 (2.76)
CL_z/F (mL/h)	65.49 (24.15)	79.88 (36.58)	52.80 (18.50)	57.18 (23.10)	46.02 (17.03)	62.65 (22.08)

* Arithmetic mean (SD) for all parameters but # was range of T_{max}.

3.4. Immunogenicity results

Of the total 71 participants, 62 (87.3%) were observed as ADA positive during the study period, including 59 of 60 participants (98.3%) receiving SSS07 and 3 of 11 subjects (27.2%) receiving a placebo. Before the administration of SSS07, 2 participants (1 in the 100 mg group and 1 in the placebo group) were found to be ADA positive. After dosing, ADA occurred initially at day 15 in the 5 mg, 15 mg, 30 mg and 50 mg groups. After day 21, participants in the other four cohorts were all found to be ADA positive. ADA constantly existed in all subjects until the last follow-up, except 2 participants (1 in the 30 mg group and 1 in the 100 mg group) who experienced the process of ADA positive-negative-positive. The period of ADA positive as observed lasted up to 1 year. Initial ADA development was at day 21 in groups with a dosage ≥75 mg, and at day 15 in cohorts ≤50 mg. The information on ADA appearance during this trial is shown in Supplementary Table 1.

3.5. TNF-α quantification results

As shown in Fig. 2, the serum concentration of TNF-α decreased immediately after the administration of SSS07. It reached a plateau stage from 4 h to 264 h in cohorts from 5 mg to 50 mg. In the 75 mg and 100 mg groups, the plateau period lasted up to 312 h. The lowest concentrations of TNF-α were close to 0 pg/mL among the 15-100 mg groups. Then, the concentration of TNF-α increased rapidly and boosted beyond the values measured at baseline. The duration of the period with TNF-α values below the baseline was from 360 h to 504 h.

4. Discussion

This study provided the first-in-human data sets of SSS07. As shown above, SSS07 was safe and well-tolerated up to 100 mg. No SAEs or deaths were found during the study. The most common AE observed was injection site reaction, which was reported in studies of other anti-TNF-α mAbs [13,14].

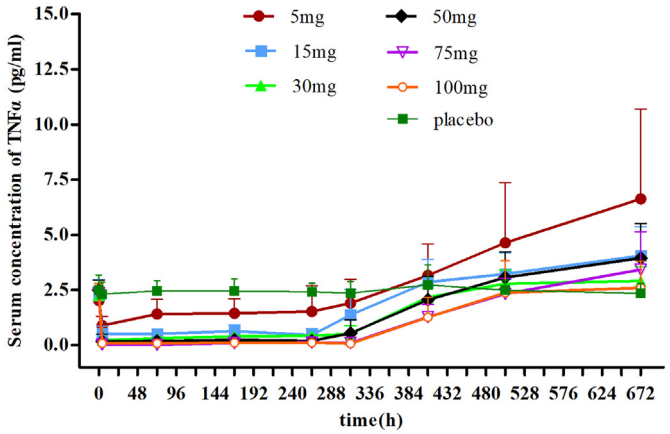

Fig. 2. Mean (SD) serum concentration-time profiles for TNF-α after a single dose subcutaneous administration of SSS07 in linear scale.

Absorption of SSS07 was slow, indicated by the T_{max} (mean) ranging from 127 to 170 h. It often took from 3 to 8 days for the subcutaneous absorption of monoclonal antibodies to reach C_{max} [15]. In the 30 mg and 50 mg groups, the average value (Mean ± SD) of T_{max} was 136.8 ± 34.0 h and 164.4 ± 50.9 h, respectively. Both values of T_{max} were slightly longer than that of adalimumab following a single 40 mg subcutaneous administration in healthy adults, which was 131 ± 56 h [16]. The C_{max} of SSS07 in the 30 mg and 50 mg groups were lower than that of adalimumab administered at 40 mg (2.70 ± 0.85 and 3.88 ± 1.24 μg/mL versus 4.7 ± 1.6 μg/mL) [17]. The apparent volume of distribution varies from 1.78 to 6.76 L, indicating that SSS07 distributed mainly in blood. The half-life of SSS07 ranged from 21.69 to 78.4 h, much shorter than that of other anti-TNF-α mAbs [17]. SSS07 was quickly cleared out 264 h post-dose, and the clearance (46.03–77.50 mL/h) was 4 to 6 times greater than adalimumab (12 mL/h) [17,18]. As a whole, SSS07 was slowly absorbed but quickly eliminated. Interestingly, SSS07 elimination presented a biphasic profile with an initial slow concentration decrease followed by accelerating rates of decline. In this trial, the rapidly reduced serum concentration of SSS07 was accompanied by ADA generation, which corresponded well to the hypothetical concentration-time curves following intravenous administration of a mAb in animals with or without ADA [19]. Furthermore, this process was verified in another animal study investigating the impact of ADA generation on the clearance of lenercept [20]. So it was reasonable to explore the impact of ADA on the PK characteristics of SSS07. After statistical analysis, results showed that compared with ADA negative participants within 672 h post-dose, ADA positive subjects had low levels of SSS07, high drug clearance, and shortened $t_{1/2z}$ (data published elsewhere) [21]. As a result, immunogenicity may cause the perturbation of PK profiles of SSS07 [22]. And large immune complexes of SSS07-ADA might form, as it was thought to be cleared more rapidly than small complexes [23]. In conclusion, PK profiles of SSS07 were favorable in ADA negative subjects.

There were significant differences in C_{max}, AUC, $t_{1/2z}$ and Vd/ F, but no difference in Clz/ F among the six groups. After multiple comparison tests, no significant differences were found in the comparison between the 30 mg and 50 mg groups in C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$. Significant differences were found in all other dose group comparisons regarding C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$. These results indicated that drug-exposure of SSS07 increased with dose escalation, but SSS07 administrated at 50 mg might not bring greater benefits compared with 30 mg administration. It was noteworthy that in the comparison between the 75 mg and 100 mg dose groups, no evidence was found that there were no significant differences in C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$. But C_{max} didn't show a significant difference in the comparison between the 50 mg and 100 mg groups. Considering that drug-exposure declined in the 100 mg group compared with the 75 mg group (Table 2), absorption saturation of SSS07 might occur in the 100 mg cohort, and it was reasonable to suggest that a dose of SSS07 of 75 mg was preferred to 100 mg. Multiple comparison tests also showed that the 5 mg group had significantly

longer $t_{1/2z}$ compared with the 30 mg group, and larger Vd/F than that the 30 mg, 50 mg, and 75 mg cohorts. Interestingly, as shown in Table 2, in the 5 mg dose group values of $t_{1/2z}$, and Vd/ F were largest compared with the other five groups. These results might indicate that neonatal Fc receptor (FcRn) mediated recycling occurred in the lower dose group, which prolonged $t_{1/2z}$ in the 5 mg dose group [24]. In addition, binding with FcRn might improve the distribution of antibodies, so in the 5 mg group, there might be a higher FcRn binding rate than that in other groups [24].

Dose-exposure proportionality analysis showed that the 90% CI for C_{max} and AUC were not fully within the ideal range. Therefore, in this research, from 5 mg to 100 mg dose levels the linear relationship between drug exposure and drug dose could not be reached, but it was improper to define a non-linear PK property of SSS07. So additional studies are needed for further investigation.

The incidence of ADA was generally high after SSS07 injection, and ADA developed in almost all participants (98.3%). So strong immunogenicity of SSS07 in humans was elicited. To our knowledge, the antigen presentation procedure was enhanced because of the formation of large immune complexes, which might result in increased immunogenicity [23]. And as documented, the period of the first occurrence of ADA ranged from 2 to 4 weeks post-dose [25]. Therefore, large complexes might form between SSS07 and TNF-α. SSS07 specific ADA initially developed within the first 28 days, and persisted in the body. The incidence of SSS07 induced ADA was high in this study, but when SSS07 is given to RA patients in the future, the incidence might decrease a lot. Because 15% of healthy volunteers receiving adalimumab were reported ADA positive but in trials conducted in RA patients the lowest ADA positive rate published was only 0.7%. Also, ADA against infliximab developed in 37% of healthy subjects, but only in 10% of RA patients [26–28]. Different assays might hamper the comparability of ADA detection among studies, and the ADA incidence of SSS07 may vary when the route of administration is changed [29,30]. Consequently, immunogenicity from rabbit derived SSS07 couldn't be recognized as higher than that of other species origin mAbs according to this study [31,32]. In some investigations, a higher dose of mAbs is associated with lower ADA incidence [33]. Though no such phenomenon was found in this study, ADA occurrence with a delay of several days in high dose groups (75–100 mg) was observed, which might be related to high dose induced immune tolerance [34]. In groups dosing from 50 to 100 mg, ADA emergence varies from 672 h to 8640 h in some participants. An individual's genetic background might contribute to this particular immune response [19]. Notably, the period of ADA occurrence in this study was in the range of that published in past studies. Pre-existing ADAs were detected in two participants (2.8%), which might be derived from the endogenous antibodies or antibodies from adaptive immune responses against environmental antigens or homologous bio-therapeutics [35]. From published research, the prevalence of pre-existing antibodies was 0.6% (3/84) in healthy volunteers [35]. It deserves attention that the immunogenicity of SSS07 was weak in animals. Different immunogenicity of SSS07 in humans and animals confirmed the view that animal studies for immunogenicity prediction of mAbs in humans were low [36].

TNF-α in serum was detected before and after SSS07 administration. In cohorts from 15 mg to 100 mg, the lowest levels of TNF-α were similar and close to 0 pg/mL. But the time of TNF-α under baseline level varied noticeably between each dosage group. Accordingly, dose relevant response might appear in the SSS07 inhibition effect. So in RA patients with an abnormally high level of TNF-α, the lowest level each dosage group of SSS07 could reach might be different. As shown in Fig. 2, the duration of TNF-α under baseline value in the 75 mg and 100 mg groups seemed almost the same, and in combination with the PK analysis, the two dosages may generate a similar TNF-α binding effect in RA patients. Nevertheless, serum TNF-α was significantly lowered in the presence of SSS07. However, in some cases, TNF-α- anti-TNF-α complex stabilized soluble TNF-α (sTNF-α) and then increased TNF-α in circulation [36]. And the level of measured TNF-α elevation was reported in etanercept treated RA patients [37]. What is more, currently available anti-TNF-α mAbs were found to prevent or slow the process of TNF-α conversion from trimers to inactive monomer, so the bioactivity of TNF-α was maintained [38]. Presumably, in this study, the quantification system was not capable of detecting the majority of TNF-α that binds to SSS07 [38]; or SSS07 down-regulated the expression of TNF-α by inactivating monocytes [39]. It could also be explained by the different biochemical and pharmacokinetic properties between rabbit and mouse resourced mAbs. Furthermore, SSS07 in vitro induced apoptosis of targeted cells through antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDCC), which might be another factor lowering TNF-α concentration. Several days later, TNF-α in serum elevated quickly and a few days later exceeded the baseline value. It was speculated that along with the descending concentration of mAbs, and constantly developed endogenous TNF-α, the serum concentration of TNF-α increased gradually. A preliminary conclusion might be the high specificity of SSS07 in the target combination. Interestingly, TNF-α arising was accompanied by ADA generation and rapid elimination of SSS07. Besides, the TNF-α inhibition effect of SSS07 lasted longer in high dosage groups (75–100 mg) and ADA occurred later in these two groups compared with the other four cohorts. These phenomena might be explained by the views that ADA and TNF-α competitively bound to the CDR of SSS07 [38]. Therefore, free TNF-α was released and levels of measurable TNF-α quickly elevated. Neutralizing antibodies (nAbs), as a result, developed mainly against SSS07. To verify this viewpoint, the serum concentration of TNF-α with ADA and without ADA occurrence was compared (Supplementary Fig. 1). As expected, ADA negative subjects experienced a longer time with TNF-α depression than ADA positive subjects. It is interesting to see that the administration of 5 mg of SSS07 (the lowest concentration) resulted in the highest serum TNF-α concentrations eventually (672 h post-dosing) compared with the other doses, which might also be considered as a reaction to the strong immunogenicity. Because it's widely accepted that initial low-dose mAbs induce a higher level of immunogenicity [40], a higher level of ADA might occur in the 5 mg dose group and competitively inhibit the TNF-α binding to SSS07, resulting in an upsurge in TNF-α concentration when compared with other groups. Based on this result, it appeared that the dosage of SSS07 ≥ 15 mg, in this study, would be preferred with lower-level ADA while there was a similar immunogenicity incidence among the six cohorts. Though a lack of up-regulation of TNF-α with infliximab was confirmed [41], it was uncertain whether there existed feedback regulation to SSS07 by increasing TNF-α production in this research. Hence, further investigations will be needed. In this study, each dosage of SSS07 suppressed TNF-α significantly and in ADA positive participants, the inhibitory function of SSS07 was weakened.

In summary, in this first-in-human study, SSS07 showed good safety and tolerance. Compared with anti-TNF-α antibodies on the market, this rabbit sourced antibody manifested favorable features in pharmacokinetics and TNF-α inhibition effect in ADA negative participants. The prevalent ADA generation suggested a high immunogenicity of SSS07, which played a pivotal role in perturbation of the PK profiles and TNF-α inhibition capability. Therefore, in further clinical investigations of SSS07, neutralizing antibodies detection is suggested and immunosuppressant application might be considered for immunogenicity control. More research needs to be conducted to drive rabbit anti-human mAbs from preclinical studies to clinical investigations successfully.

5. Conclusion

Single-dose subcutaneous administration of SSS07 was safe and well-tolerated in healthy Chinese adults. Doses and exposure of SSS07 from 5 mg to 100 mg could not be regarded as nonlinear based on dose-exposure proportionality analysis. A high incidence of anti-drug

antibodies may have an impact on the pharmacokinetics profile and interfere with TNF-α binding capability of SSS07. Immunogenicity of SSS07 was strong and the profiles varied greatly between animals and humans.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.105807.

Authors' individual contributions

Yi Fang, Haifeng Song, Zhanguo Li, and Shi Chen contributed to the study protocol establishment. Yi Fang, Tao Tao and Quanrui Wu supervised the whole study. Qian Wang was responsible for drug administration and bio-samples collection. Daoli Jiang was responsible for bio-samples management. Yitong Wang and Chang Liu contributed to the data files check. Xinyao Xie, Min Han, and Nana Xi contributed to the drug qualification process. Qi Wang, Feng Su, Jiaxue Wang and Tan Zhang contributed to the literature search, data analysis, and manuscript writing. All co-authors approved the final manuscript.

Funding information

This study was funded by Shenyang Sunshine Pharmaceutical Company Limited, Shenyang city, Liaoning province, China..

Data availability statement

Data are available on request from the authors. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors have no conflicts of interest that are directly relevant to the content of this study.

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