# Safety and immunogenicity from a Phase I trial of inactivated severe acute respiratory syndrome coronavirus vaccine

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**Background: Emergence of severe acute respiratory syndrome (SARS) from the winter of 2002 to the spring of 2003 has caused a serious threat to public health.**
**Methods: To evaluate the safety and immunogenicity of the inactivated SARS coronavirus (SARS-CoV) vaccine, 36 subjects received two doses of 16 SARS-CoV units (SU) or 32 SU inactivated SARS-CoV vaccine, or placebo control.**
**Results: On day 42, the seroconversion reached 100% for both vaccine groups. On day 56, 100% of participants in the group receiving 16 SU and 91.1% in the group receiving 32 SU had seroconverted. The geometric mean titre of neutralizing antibody peaked 2 weeks after the second vaccination, but decreased 4 weeks later.**
**Conclusion: The inactivated vaccine was safe and well tolerated and can elicit SARS-CoV-specific neutralizing antibodies.**

## Introduction

Severe acute respiratory syndrome (SARS), the newly emerging respiratory illness caused by SARS coronavirus (SARS-CoV), has been a great threat to public health worldwide [1,2]. From November 2002 to the end of July 2003, 8,096 cases of SARS were reported, and 774 of the infected patients died [3]. During the winter season of 2003–2004, four sporadic SARS cases were identified in Guangdong province China, in which no transmission in hospital or in the community has been identified. Meanwhile, from October 2003 to April 2004, there were three SARS laboratory accidents reported in Taiwan, Singapore and Beijing. In the latter case, nine SARS cases have been confirmed to be due to exposure to the same infectious origin. Several studies indicated that civet cat and fox might be the animal reservoir for SARS-CoV [4,5]. The SARS threat to public health is far from eradication.

To prevent the future reoccurrence of SARS, numerous groups are dedicated to SARS vaccine research and development through different pathways including inactivated, viral vector, recombinant and DNA vaccines [6–11]. Serum-neutralizing antibodies against SARS-CoV have been successfully induced in experimental animals by several candidate vaccines. In May 2004, the inactivated SARS-CoV vaccine (ISCV) developed by Sinovac Biotech Co. Ltd (Beijing, China) was authorized by the China State Food and Drug Administration (SFDA) to enter a Phase I clinical trial for the first time worldwide. A total of 36 healthy adults were enrolled into this investigation conducted in Beijing. Here we report the results of the safety and immunogenicity of ISCV from this randomized, double-blind, placebo-controlled clinical study.

## Materials and methods

### Eligible subjects

A total of 36 healthy adults, 18 males and 18 females, aged between 21 and 40 were enrolled from the Beijing metropolitan area. All volunteers were requested to give their written, informed consent, in conformity with international medical ethics, before the start of trial and all relevant documents were approved by the ethical review committee of the Chinese–Japanese Friendship Hospital. The healthy physical conditions of the candidates were evaluated by medical history, physical examination and routine laboratory tests. Candidates were excluded if they showed any of the following: serum antibody-positive for SARS-CoV, HCV or HIV, serum HBsAg-positive, chronic diseases, history of neuropathy or mental disorders, immunosuppression due to illness or treatment, abnormalities on haematology, clinical chemistry, routine urinalysis, chest X-ray or electrocardiograph, fever >37°C (by axillary) on the day of vaccination, or vaccination or taking of experimental drugs during the past 2 months. Female volunteers who were pregnant, lactating or refused to use a reliable method of contraception during the study were also excluded.

### Vaccines

The inactivated SARS-CoV vaccine in this study was developed by Sinovac Biotech Co. Ltd, Beijing, China, and was derived from a clinical strain (Sino 3) originally isolated from a pharyngeal swab of a definitely diagnosed SARS patient from Beijing, 2003 (GenBank accession number AY485278). In brief, SARS-CoV Sino3 strain was propagated in Vero cells (obtained from the American Type Culture Collection, that were free of adventitious agents at passage 147 and inactivated by β-propiolactone. After full inactivation was verified, the harvests were purified. The content of SARS-CoV antigen in bulk was measured by a passive indirect haemagglutination assay: briefly, aldehyde-pretreated O-type human erythrocytes were sensitized with purified rabbit anti-SARS-CoV immunoglobulin (IgG). ISCV bulk was fourfold series diluted in 96-well plates and incubated with the red blood cells at room temperature for 1 h, taking the dilution value of the well showing 50% haemagglutination as the haemagglutination titre of the tested ISCV. The content of ISCV antigen was described in SARS-CoV units (SU), defined as the reciprocal of the haemagglutination titre of the tested ISCV. A previously well titrated and freeze-dried batch of SARS antigen reference that showed 75 SU was used as the control. The SU of the tested ISCV was standardized with that of SARS antigen reference. The ISCV was adsorbed to aluminium hydroxide and packed into pre-filled syringes in sterile saline without preservative at the following antigen content: lot number 20031126-1 16 SU/ml, and lot number 20031216-1 32 SU/ml. The placebo vaccine for the control consisted of only sterile saline and aluminium hydroxide. The safety was evaluated and the dose range was selected on the basis of preclinical trials in mice, rats and rhesus monkeys, which clearly showed that an aluminium hydroxide adjuvant was needed. All manufacturing processes fulfilled the requirements for vaccine development and manufacture issued by the China SFDA.

### Study design and procedure

The study was designed as a randomized, double-blind and placebo controlled study. The trial was conducted in the Beijing China–Japan Friendship Hospital in full accordance with China SFDA and World Health Organization (WHO) good clinical practice guidelines and overseen by a data and safety monitoring board. The protocol of the study was peer-reviewed and approved by the Hospital Ethics Committee.

Subjects were divided into three groups randomly, receiving 16 SU or 32 SU ISCV or placebo control vaccine. Each group contained 12 participants. An off-site statistician generated randomization codes by computer for two dose groups, with two ISCV and one control per block. The sponsor masked the vaccines with sequential study numbers according to the randomization codes list and kept the randomization codes list until opening the blind. Subjects were allocated study number sequentially, and thus randomly allocated trial vaccine. Vaccines were administered by intramuscular injection into the deltoid muscle 28 days apart. In practice, a small group of subjects was randomly allocated to receive the various vaccines and providing that no serious vaccine-related events occurred during the first week, the rest subsequently finished the vaccination processes.

On the day of each vaccination, the temperatures of the subjects were taken immediately before and 30 min after each vaccination. For the following 3 days, subjects were asked to come back to hospital for clinical observation. Local reactions (pain, erythema and swelling), systemic reactions (fever, headache, malaise, fatigue, nausea and vomiting) and any other symptoms were recorded on the case report form. Temperatures were measured each day by axillary. After day 4 subjects were asked to use a log report form to record local and system symptoms. The adverse events were graded and evaluated based on the guidelines of the National Institutes of Health (NIH) and WHO [12].

### Laboratory assays

Blood was collected immediately before each immunization (day 0 and day 28) and on days 7, 14, 35, 42

and 56, and according to the schedule the last blood samples were taken on day 210. Whole blood or serum samples were prepared routinely and employed for further clinical investigations, including alanine aminotransferase (ALT), blood urea nitrogen, creatinine, total bilirubin, direct bilirubin, white blood cell count, red blood cell count, haemoglobin and platelet count. Urine was collected at the same time points to be applied for routine urinalysis. $CD4^+$ and $CD8^+$ T-cell counts were evaluated at days 0, 28 and 56.

### Immunogenicity assays

The serum anti-SARS antibodies were measured by a neutralization assay that was conducted in the BSL-3 laboratory in the National Institute for Communicable Disease Control and Prevention, Chinese Centre for Disease Control and Prevention. The reference virus in neutralization assays was SARS-CoV strain Sino 1, which has been well characterized for its biological characteristics, including antigenicity, immunoreactivity, morphology and complete genome sequence (GenBank accession number AY485277). Briefly, 100 µl twofold serial-diluted sera, from 1:2 to 1:1,024, were added to an equal volume of virus (diluted to 100 50% cell culture infectious doses [$CCID_{50}$]/0.1 ml) and incubated for 1 h at 37°C before being added to confluent monolayers of Vero cells in 96-well plates and incubated at 37°C in a 5% $CO_2$ incubator. A previously well titrated batch of serum from a convalescent SARS patient in Zhangjiakou, Hebei, China was used as a positive control. This reference serum showed 1:57.2 geometric mean titre (GMT) based on the results from 21 independent assays, so the neutralizing efficacy was defined as 57.2 U. One unit is described as the reciprocal of the dilution of sera that can neutralize 100 $CCID_{50}$ of SARS-CoV. A panel of sera collected from 30 healthy blood donors was used as negative control, which was previously tested to be SARS-CoV-antibody-negative with ELISA and neutralization assays. Meanwhile, the reference virus was re-titrated for its infectivity ($CCID_{50}$/0.1 ml) in parallel. Four days after challenge, viral cytopathic effect (CPE) on the infected cells was monitored. The dilution of serum that completely prevented CPE in 50% of the wells was calculated by the Reed–Muench formula [13]. For the purpose of calculation, neutralizing antibody titres >2 U were assigned an arbitrary value of 2 U. The serum-neutralizing antibody titre after vaccination with a four-fold increase was considered as positive for seroconversion. For each sample, the neutralizing effects were tested at least three times. The tests were taken as valid only when the neutralizing efficacy of the reference serum was in the range of 34–68 U and the infectivity of the reference virus was in the range of 70–160 $CCID_{50}$/0.1 ml. Serum SARS-CoV-specific IgG was evaluated by a commercial ELISA kit (SARS Coronavirus IgG Antibody Diagnostic Kit, BGI-GBI Biotech Co Ltd, Beijing, China). Briefly, 100 µl twofold serial-diluted sera, starting from 1:2 to 1:1,024, were employed into the ELISA wells and processed according to the manufacturer's protocol. The criteria of SARS-Cov IgG seroconversion were the same as those of neutralizing antibody described above.

### Statistical analysis

Statistical analysis was performed by SAS software (SAS, Cary, NC, USA). The incidence of local and systemic events was compared between groups using the Fisher's exact test. Fisher's exact test was also used to compare seroconversion rates. The neutralizing antibody titres of subjects were tested first by normal distribution analysis. If the titres were normally distributed, the GMT between groups was compared by means of Student's *t*-test. If the titres were not normally distributed, the Mann–Whitney test was used. A *P*-value of $\leq 0.05$ was considered significant.

## Results

From May to October 2004, 36 healthy adults from the Beijing metropolitan area were enrolled into a Phase I clinical trial for an inactivated SARS vaccine developed by Beijing Sinovac Co. Ltd. Each group contained six males and six females without significant difference in age. All subjects completed the designed processes, including immunization, adverse events monitoring and serological assessment. None withdrew from the study.

### Safety

No immediate adverse reactions were reported within the first 2 h after immunization. Within 72 h after vaccination, local mild pain occurred in five individuals given 16 SU, one given 32 SU and one in the placebo group, without statistical difference among the groups (Table 1). Two participants in the 16 SU group had local erythema. One subject in the 16 SU group experienced local itching. All symptoms were mild and resolved within 48 to 72 h. Diarrhoea was noted in three individuals given 16 SU, one in the group given 32 SU and two in the placebo group, without significant differences (Table 1). The most severe diarrhoea an individual described was three times in 1 day and the symptoms resolved in all affected participants within 24 h. Abdominal pain was reported by one participant receiving 16 SU, two receiving 32 SU and one receiving placebo (Table 1). Other symptoms, such as slight fever, headache, fatigue, arthralgia, myalgia, angina and dysphoria, were also noted in very few

**Table 1.** Number and incidence of adverse events after two dose injections

| Event | 16 SU ISCV (*n*=12) | | | 32 SU ISCV (*n*=12) | | | Placebo (*n*=12) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Dose 1 | Dose 2 | Total* | Dose 1 | Dose 2 | Total* | Dose 1 | Dose 2 | Total* |
| **Local events** | | | | | | | | | |
| Pain | | | | | | | | | |
| Mild | 5 (41.7) | 2 (16.7) | 5 (41.7) | 2 (16.7) | 1 (8.3) | 2 (16.7) | 1 (8.3) | 0 | 1 (8.3) |
| Moderate | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Severe | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Erythema ≥10 mm | 2 (16.7) | 0 | 2 (16.7) | 0 | 0 | 0 | 0 | 0 | 0 |
| Itch | 1 (8.3) | 0 | 1 (8.3) | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | – | – | 8 (66.7) | – | – | 2 (16.7) | – | – | 2 (16.7) |
| **Systemic events** | | | | | | | | | |
| Fever ≥37.1°C | 0 | 0 | 0 | 0 | 1 (8.3) | 1 (8.3) | 0 | 0 | 0 |
| Headache | 0 | 0 | 0 | 1 (8.3) | 0 | 1 (8.3) | 0 | 0 | 0 |
| Vertigo | 0 | 0 | 0 | 0 | 0 | 0 | 1 (8.3) | 0 | 1 (8.3) |
| Fatigue | 0 | 0 | 0 | 1 (8.3) | 0 | 1 (8.3) | 0 | 0 | 0 |
| Arthragia | 0 | 0 | 0 | 1 (8.3) | 0 | 1 (8.3) | 1 (8.3) | 0 | 1 (8.3) |
| Myalgia | 1 (8.3) | 0 | 1 (8.3) | 0 | 0 | 0 | 1 (8.3) | 0 | 1 (8.3) |
| Angina | 1 (8.3) | 0 | 1 (8.3) | 0 | 0 | 0 | 1 (8.3) | 0 | 1 (8.3) |
| Abdominal pain | 1 (8.3) | 0 | 1 (8.3) | 1 (8.3) | 1 (8.3) | 2 (16.7) | 0 | 1 (8.3) | 1 (8.3) |
| Diarrhoea | 2 (16.7) | 1 (8.3) | 3 (25.0) | 1 (8.3) | 0 | 1 (8.3) | 1 (8.3) | 1 (8.3) | 2 (16.7) |
| Dysphoria | 1 (8.3) | 0 | 1 (8.3) | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | – | – | 7 (58.3) | – | – | 7 (58.3) | – | – | 7 (58.3) |

*Number of subjects with an adverse event among those vaccinated with two doses of vaccine. ISCV, inactivated severe acute respiratory syndrome coronavirus vaccine; SU, severe acute respiratory syndrome coronavirus units.

cases, which were distributed sporadically over all three groups. All symptoms resolved within 24 h.

Clinical laboratory assays revealed that 32 participants maintained normal ALT values during the course of the clinical trial, but four subjects showed transient abnormalities. Three participants receiving 32 SU had grade 1 increases in serum ALT on day 42 (WHO and NIH 1.25–2.5× upper limit of normal); one subject in the placebo group had a grade increase. On day 56, one subject with increased ALT had decreased to the normal range, one maintained a similar level to day 42, and the other increased to the range of a grade 2 reaction, whereas the increased ALT in the subject in the placebo group decreased to the range of grade 1. All the increased ALT reverted to normal levels within 1–2 weeks in follow-up observation. Statistical analyses of incidence of this phenomenon between the 32 SU and placebo groups showed no significant difference ($P$=0.590). The values of both the total and direct bilirubin of all participants remained in the normal ranges. Other clinical indexes, including the routine haematology, $CD4^+$ and $CD8^+$ T-cell counts, renal functions and routine urinalysis assays did not reveal remarkable abnormalities in any of the enrolled individuals.

## Immunogenicity

Before vaccination all subjects were confirmed to be negative for serum SARS-CoV antibody with ELISA. The criterion set for determining the immunogenicity of ISCV was 85% of individuals showing seroconversion on the 56th day after vaccination. Table 2 shows that 100% participants (12/12) in the 16 SU group and 91.1% (11/12) in 32 SU group had seroconverted by day 56, whereas all sera collected from the individuals in the placebo group simultaneously were negative (less than 1:2 dilution). The seroconversion ratios both in low- and high-dosage groups were higher than 85%, indicating the success of ISCV to induce specific neutralizing antibodies in human beings.

The neutralizing antibody was first detected in the subjects (2/12) from the group receiving 16 SU by day 14 after the first injection, but with low titre (Table 3; Figure 1). One week after the second injection on day 28, more individuals both in the group receiving 16 SU (6/12) and that receiving 32 SU (7/12) had seroconverted. On day 42, all participants in the two ISCV groups had become seropositive, while the average titres were much higher (Table 3; Figure 1). The seroconversion frequency and the antibody titres were remarkably improved after the second vaccination. Four weeks after the second injection most of the subjects (23/24) maintained seropositivity, but the average antibody titres were lower than that on day 42 (Table 3; Figure 1). One case in the group 32 SU became seronegative by day 56 (neutralizing titre 1:5). However, this subject showed only 1:8

**Table 2.** Seroconversion analysed by ELISA at different times after vaccination

| Day | 16 SU group (*n*=12) | | 32 SU group (*n*=12) | | Placebo (*n*=12) | |
|---|---|---|---|---|---|---|
| | Seroconversions, *n* | Rate, % | Seroconversions, *n* | Rate, % | Seroconversions, *n* | Rate, % |
| 0 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| 7 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| 14 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| 28 | 3 | 25.00 | 2 | 16.67 | 0 | 0.00 |
| 35 | 7 | 58.33† | 6 | 50.00* | 0 | 0.00 |
| 42 | 12 | 100.00† | 10 | 83.33† | 0 | 0.00 |
| 56 | 12 | 100.00† | 9 | 75.00† | 0 | 0.00 |
| 210 | 11 | 91.67† | 8 | 66.67† | 0 | 0.00 |

*Statistical difference $P<0.05$. †Statistic difference $P<0.01$. SU, severe acute respiratory syndrome coronavirus units.

**Table 3.** The seroconversion analysed by neutralization assays at different times after vaccination

| Day | 16 SU group (*n*=12) | | 32 SU group (*n*=12) | | Placebo (*n*=12) | |
|---|---|---|---|---|---|---|
| | Seroconversions, *n* | Rate, % | Seroconversions, *n* | Rate, % | Seroconversions, *n* | Rate, % |
| 0 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| 7 | 0 | 0.00 | 0 | 0.00 | 0 | 0.00 |
| 14 | 2 | 16.67 | 0 | 0.00 | 0 | 0.00 |
| 28 | 0 | 0.00 | 1 | 8.33 | 0 | 0.00 |
| 35 | 6 | 50.00* | 7 | 58.33† | 0 | 0.00 |
| 42 | 12 | 100.00† | 12 | 100.00† | 0 | 0.00 |
| 56 | 12 | 100.00† | 11 | 91.67† | 0 | 0.00 |

*Statistical difference $P<0.05$. †Statistic difference $P<0.01$. SU, severe acute respiratory syndrome coronavirus units.

neutralizing titre on day 42, which just met the criteria of seroconversion. Analyses of the GMTs of specific neutralizing antibody for SARS-CoV from these two dosage groups revealed the same kinetics, indicating that both dosages of ISCV induced similar humoral immunoresponses in humans. The titres of neutralizing antibodies peaked 2 weeks after the second vaccination, but started to drop 4 weeks later.

Gender-grouping analyses of GMT values from the two vaccination groups showed similar kinetics. The numbers of individuals that seroconverted after the second vaccination were the same for males and females on days 35 and 42, and similar on day 56 (11:12). However, the female GMT value was statistically significantly higher than that of the males on day 42 (*t*-test $P=0.025$). On day 56, the female GMT value had decreased to a similar level as the males (*t*-test $P=0.813$). Age grouping revealed similar kinetics of the numbers of seroconverters and GMT of neutralizing antibody. On day 35, the number of individuals that seroconverted and the level of GMT of neutralizing antibody in the participants between 21–30 years old were lower than those in the elder group, but without significant differences (seroconversion $P=0.444$; GMT $P=0.528$). On day 42, the numbers of seroconverted individuals were the same. However, GMT in the younger group (<31 years old) remained still lower than in the elder group.

In parallel, SARS-CoV-specific antibodies of all subjects were evaluated with a commercial ELISA kit. SARS-CoV antibodies were positive in five subjects (3/12 in the 16 SU group and 2/12 in the 32 SU group) on day 28 and peaked on day 42, in which all participants in the group receiving 16 SU (12/12) and 83.33% of participants receiving 32 SU (10/12) were seropositive (Table 2). Four weeks after the second injection, most subjects (21/24) maintained seropositivity. Even 210 days after vaccination, most subjects receiving ISCV remained seropositive (11/12 in 16 SU group and 8/12 in 32 SU group). No statistical difference has been identified between the 16 SU group and the 32 SU group. Comparing the seroconvertion results evaluated by neutralizing test and ELISA in 24 subjects receiving ISCV did not reveal statistical differences at each testing time.

## Discussion

Overall, the inactivated SARS-CoV vaccine was well tolerated in humans. No severe adverse reaction (grade 3) was described. Three subjects in the 32 SU

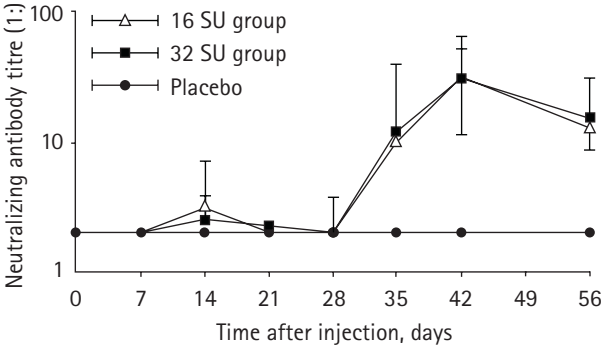
group and one in the placebo group showed transient increases of serum ALT, which resolved within the following week. Other indexes reflecting liver functions stayed within the normal ranges. We believe that the mild ALT adverse reactions observed in this study were not ISCV-related. Although a group has reported that they observed severe liver inflammation when a recombinant vaccine, created by genetically modifying a pox virus to produce SARS-CoV spike (S) protein, was tested in ferrets [14], our previously performed pathological study on monkeys [15] and another study [16] did not find any evidence of liver damage. More research on the influences of SARS-CoV on liver functions in animal experiments are needed to address the potential liver damage of SARS virus vaccine candidates.

The data presented in this study demonstrate that SARS-specific neutralizing antibodies could be efficiently elicited by the tested ISCV. In line with many previous studies [17–19], none of the participants in this trial showed any pre-existing serum antibody to SARS-CoV. Therefore, we believe that the immunoreactivities to SARS-CoV in the subjects are vaccination-specific. Although the number of participants was relatively small, the seroconversion rates of the two dosage groups satisfy the criteria set for this Phase I clinical trial for immunogenicity assessment of ISCV. The GMT values in younger individuals were slightly lower than those of the older ones, but statistical analyses did not reveal any significant difference. SARS infections in the 2003 outbreak had a remarkable age-related distribution; children and teenagers were rarely affected [20,21]. More broad age grouping in clinical trials is needed to address the possible age difference, especially in young people. Although gender difference in GMT value was observed on day 42, the real meaning is hard to explain on the basis of the limited data in this trial.

Previous studies on several animal coronaviruses suggest not only humoral but also cellular immunity have roles in protection against virus infection [22,23]. However, neutralizing antibody in body fluids seems essential for counteracting SARS infection. Retrospective laboratory assays confirm that a high percentage of convalescent SARS patients possess detectable neutralizing antibody [18,26]. Epidemiological data in 2003 reveal that no reinfection of SARS was reported during the 8-month worldwide epidemic [27]. Recently a DNA vaccine encoding the S glycoprotein of the SARS-CoV has been reported to be able to induce mice to generate T-cell and neutralizing antibody responses. However, the protection was mediated by a humoral but not T-cell-dependent immune mechanism through T-cell depletion and passive transfer of purified IgG [28]. In a mouse model, the neutralizing antibody elicited by primary infection can protect from reinfection. Subsequently, passive transfer of this immune serum to vaccine-naive mice can prevent SARS-CoV replication in the respiratory tract [29]. Immunizing monkeys with adenoviral-vector-based recombinant viruses that express S, membrane and nucleocapsid proteins of SARS-CoV also induced specific neutralizing antibody response [30]. These results indicate that neutralizing antibody in humans can provide protection against SARS-CoV.

During our pre-clinical study of ISCV, we have tested serum neutralizing titres from 87 convalescent sera of clinically confirmed SARS patients from different areas in China, illustrating that the GMT is 61 U [31]. Using the same laboratory standard and methodology, the GMT values on day 42 in the groups of 16 SU and 32 SU are 31.1 U and 30.9 U, respectively, half of those in the convalescent SARS patients. It is generally accepted that the antibody titres elicited by vaccination are usually lower than that induced by natural infection. Although specific criteria for assessing the efficacy of SARS vaccine have not been defined, neutralizing antibody titres at this level after vaccination could be considered significant for protection against SARS-CoV infection. Further trials are needed to establish the criteria for evaluating the efficacy of SARS vaccine and to optimize vaccine dose and schedule.

**Figure 1.** The change in neutralizing antibody titre after vaccination




Twelve subjects in each group received 16 SU or 32 SU inactivated severe acute respiratory syndrome (SARS) coronavirus vaccine (ISCV) or placebo vaccine at day 0 and day 28, respectively. The sera were collected and tested for the presence of SARS-specific antibody by neutralization assay. The neutralizing antibody titre shown in the figure was the geometric mean titre of 12 subjects in each group.

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