Randomized, double-blinded and placebo-controlled phase II trial of an inactivated SARS-CoV-2 vaccine in healthy adults

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Summary: An inactivated SARS-CoV-2 vaccine was evaluated in a randomized, double-blinded and placebo-controlled phase II trial involving 750 healthy adults. Serological detection of neutralizing antibodies and ELISA antibodies and clinical observation indicated the immunogenicity and safety of the vaccine.

Abstract

Background

We evaluated an inactivated SARS-CoV-2 vaccine for immunogenicity and safety in adults aged 18-59 years.

Methods

In this randomized, double-blinded and controlled trial, healthy adults received a medium (MD) or a high dose (HD) of the vaccine at an interval of either 14 days or 28 days. Neutralizing antibody (NAb) and anti-S and anti-N antibodies were detected at different times, and adverse reactions were monitored for 28 days after full immunization.

Results

A total of 742 adults were enrolled in the immunogenicity and safety analysis. Among subjects in the 0, 14 procedure, the seroconversion rates of NAb in MD and HD groups were 89% and 96% with GMTs of 23 and 30, respectively, at day 14 and 92% and 96% with GMTs of 19 and 21, respectively at day 28 after immunization. Anti-S antibodies had GMTs of 1883 and 2370 in MD and 2295 and 2432 in HD group. Anti-N antibodies had GMTs of 387 and 434 in MD group and 342 and 380 in HD group. Among subjects in the 0, 28 procedure, seroconversion rates for NAb at both doses were both 95% with GMTs of 19 at day 28 after immunization. Anti-S antibodies had GMTs of 937 and 929 for MD and HD group, and anti-N antibodies had GMTs of 570 and 494 for MD and HD group, respectively. No serious adverse events were observed during the study period.

Conclusion

Adults vaccinated with inactivated SARS-CoV-2 vaccine had NAb as well as anti-S/N

antibody, and had a low rate of adverse reactions.

Clinical trials registration

NCT04412538.

Key words:

Inactivated SARS-CoV-2 vaccine, phase II trial, immunogenicity, safety

The global pandemic of coronavirus disease 2019 (COVID-19) induced by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a new member of the coronavirus family, caused active responses from governments and organizations worldwide [1-4] and led to projects for the development of approximately 300 candidate vaccines (assessed at *ClinicalTrials.gov* as of Sep. 29, 2020). Overall, more than 10 different types of vaccines have entered into clinical trials [5]. However, without a systematic understanding of this viral pathogen and its pathogenesis and immunologic features [6, 7], evaluations of the immunity and safety of these vaccines, which were developed through various technologies, are more dependent upon an analysis of serologic detection and adverse reaction data collected from subjects at different stages of clinical trials. Our previous work developed an inactivated SARS-CoV-2 vaccine with S-antigen and N-antigen exposure that elicited not only neutralizing antibodies but also antibodies against N-antigen that are associated with specific protective effects in a rhesus model under viral challenge. This vaccine entered clinical testing (Number: 2020L00020 from the Chinese State Food and Drug Administration (SFDA); ClinicalTrials registration number NCT04412538), and a phase I trial was successfully completed with recognized safety of the vaccine. Low rates of mild adverse reactions and unchanged levels of 48 cytokines in the serum of immunized subjects were observed, and the antibody response was higher than 95% at medium and high doses (manuscript in submission). In the work presented here, we further evaluated the immunity and safety of this vaccine in a phase II trial in which we enrolled 750 healthy adults 18-59 years of age for immunization at an interval of either 14 days (0, 14 procedure) or 28 days (0, 28 procedure), and for each procedure, either a medium or a high dose was administered. The trial was a

randomized, double-blinded and placebo-controlled trial that showed that two doses of the vaccine elicited effective antibody responses, including neutralizing antibodies, with a low rate of mild adverse reactions.

Material and method

Inactivated vaccine

The SARS-CoV-2 inactivated vaccine was developed by the Institute of Medical Biology (IMB), Chinese Academy of Medical Sciences (CAMS). Briefly, the virus strain, named KMS-1 (GenBank No: MT226610.1), which was isolated from a patient with a definite case of COVID-19 who was hospitalized at Yunnan Hospital of Infectious Diseases, was inoculated into Vero cells (World Health Organization (WHO)) cultured in Dulbecco's modified Eagle's medium (DMEM; Corning, NY, USA) containing 5% fetal bovine serum (FBS; HyClone, Logan, USA) for production in an environment that met Biosafety Level (BSL) requirements. To disrupt the viral membrane, formaldehyde (v:v=1:4000) was added to the harvested viruses for 48 h. Then, for purification, chromatography and concentration were performed. For further inactivation, beta-propiolactone (v:v=1:2000), which is known to modify viral RNA through alkylation for virus inactivation, was added, followed by a second purification using the same protocol. The quality of the vaccine was evaluated according to Good Manufacturing Practice (GMP) requirements. The vaccine contained 100 or 150 enzyme-linked immunosorbent assay (ELISA) units (EU; the viral antigen concentration determined by ELISA) of inactivated viral antigen adsorbed to 0.25 mg of $Al(OH)_3$ adjuvant and

suspended in 0.5 ml of buffered saline in each dose. The placebo contained only Al(OH)$_3$ in an equivalent amount of buffer.

Study design and participants

This randomized, double-blinded and adjuvant (Al(OH)$_3$)-controlled trial was performed to evaluate the immunogenicity and safety of an inactivated SARS-CoV-2 vaccine. The protocol was reviewed and approved by the Ethics Committee of the Center for Disease Prevention and Control (CDC) of Yunnan Province. An independent data safety monitoring board (DSMB) was established for monitoring the safety data specifically related to the suspension and termination of the trial during the study and had regular meetings before starting the trial, as 50% enrollment reached for safety data reviewing and the close of trial. According to the Declaration of Helsinki and Good Clinical Practice, the trial was conducted at the CDCs of Mile and Gejiu County of Yunnan Province, where the pandemic events of COVID-19 were recorded (Supplemental Fig. 1). Healthy volunteers aged 18 to 59 years were eligible for enrollment with written informed consent. The inclusion and exclusion criteria are listed in the supplementary appendix. A total of 750 enrolled participants, with 375 in Gejiu county and 375 in Mile county, were allocated to the procedures with an interval of 14 days or 28 days between two inoculations, respectively. Furthermore, the subjects in each procedure were randomly assigned at a 2:2:1 ratio to receive a medium dose (containing 100 EU viral antigen) or a high dose (containing 150 EU viral antigen) of the SARS-CoV-2 vaccine or the placebo as a control. Blood samples were collected from the enrolled participants at days 0 (baseline), 14 and 28 (0, 14 procedure), and day 28 (0, 28 procedure) following the boost immunization to evaluate the potential immunogenicity of the vaccine at different time

points. The solicited and unsolicited adverse events (AEs), if any, were recorded within a period of 0-7 days following the 1st inoculation and 0-28 days after the second inoculation. Study staff visited participants on site to monitor their health status and determine whether the participants needed to seek medical care when they returned to submit their safety records or provide blood samples.

Endpoints of the clinical trial

The primary endpoints were the seroconversion rates of the anti-SARS-CoV-2 neutralizing antibody and ELISA IgG antibody at days 14 (0, 14 procedure) and 28 (0, 28 procedure), respectively, after the boost immunization. There were 2 secondary endpoints: (1) the total AE rate from 0 to 7 days following each inoculation and from 0 to 28 days after the boost immunization, and (2) the geometric mean titer (GMT) profiles of the anti-SARS-CoV-2 neutralizing antibody and ELISA IgG antibody at days 14 and 28 (0, 14 schedule) and day 28 (0, 28 schedule) following the boost immunization.

Safety assessment

AEs included local and systemic reactogenicity signs and symptoms from 0 to 7 days following each inoculation and from 0 to 28 days after the boost immunization. Serious adverse events (SAEs) were recorded for a period of 12 months following the boost immunization. The severity of all the AEs was graded in accordance with the Guideline for Grading Adverse Events in Clinical Trials of Preventive Vaccines newly issued by the National Medical Products Administration (NMPA) in 2019.

Neutralizing antibody test

The neutralizing antibody assay was performed via microtitration in 96-well plates. Briefly, heat-inactivated serum was diluted and coincubated with live virus (100 $lgCCID_{50}$/well) for 2 h at 37°C, followed by the addition of Vero cells (10^5/mL), and the mixture was incubated at 37°C in 5% CO_2 for 7 days. The cytopathic effects (CPEs) were observed and assessed to determine the neutralizing antibody titer. The GMTs of neutralizing antibodies were measured. Antibody titers of ≥4 were considered positive.

ELISA

Enzyme-linked immunosorbent assays (ELISAs) were conducted with antibodies against the S-protein and N-protein (5 µg/well; Sanyou Biopharmaceuticals Co., Ltd., Shanghai, China) coated onto 96-well ELISA plates (Corning, NY, USA) at 4°C overnight. The plates were blocked with 5% bovine serum albumin (BSA), incubated with serum samples and visualized with a horseradish peroxidase (HRP)-conjugated antibody (Abcam, MA, USA) and tetramethylbenzidine (TMB) substrate (Solarbio, Beijing, China). The absorbance of each well at 450 nm was measured using an ELISA plate reader (Gene Company, Beijing, China). Diluted serum samples with an optical density (OD) value greater than or equal to 2.1-fold the OD value of the negative control were defined as positive. The endpoint titer (ET) was defined as the highest dilution of a positive serum sample. The geometric mean endpoint titer (GMET) was calculated as the geometric mean of the ETs of positive serum samples

from the same group. Similar to neutralizing antibodies, seroconversion was defined as seronegative subjects becoming seropositive after immunization.

Results

Participant flow

A total of 1130 adults were recruited for this phase II trial in two counties, Gejiu and Mile, and 750 were eligible for participation (Fig. 1), with 375 in Gejiu for the 0, 14 procedure and 375 in Mile for the 0, 28 procedure. Eligible participants from each county were randomly assigned to the medium- and high-dose groups and the placebo control group at a ratio of 2:2:1 (Fig. 1). In June, immunization with the vaccine was performed at two sites, followed by clinical observation for 28 days and the collection of blood samples at days 14 and 28 for the 0, 14 procedure or at day 28 only for the 0, 28 procedure. A total of 374 subjects who underwent the 0, 14 procedure were entered into the full analysis set (FAS), and 372 were entered into the per protocol set (PPS), with 148 (98.7%) in the medium-dose group and 149 in the high-dose group. A total of 371 and 370 subjects who underwent the 0, 28 procedure were entered into the FAS and PPS, respectively (Fig. 1). The demographic characteristics of the PPS are described in Table 1.

Assessment of immunity elicited by the vaccine in the two immunization procedures

The primary immunologic outcome of this phase II trial of an inactivated SARS-CoV-2 vaccine was the neutralizing antibody response against SARS-CoV-2 in the two dose groups with different immunization procedures. The results suggested that in the immunization procedure with an interval of 14 days, the compared to those in the placebo control group

seroconversion rates in the medium- and high-dose groups were 89% and 96%, respectively, with GMTs of 23 and 30, respectively, at day 14 after immunization (Fig. 2a), and 92% and 96% with GMTs of 19 and 21, respectively, at day 28 after immunization (Fig. 2a). In the immunization procedure with an interval of 28 days, the seroconversion rates in the medium- and high-dose groups were both 95%, with GMTs of 19, at day 28 after immunization (Fig. 2b). The secondary immunologic outcome of this study was the ELISA antibody response elicited by the inactivated vaccine designed by our specific technical strategy for presenting viral S- and N-antigens. The results of detection using the plate coated with S protein suggested that anti-S antibody showed seroconversion rates that were similar to those of neutralizing antibody, with GMTs of 1883 and 2370 in the medium- and high-dose groups, respectively, for the 0, 14 procedure at day 14 after immunization and higher seroconversion rates with GMTs of 2295 and 2432 at day 28 after immunization (Fig. 2a). The detection of anti-N antibody suggested approximately 60% seroconversion with GMTs of 387 and 434 at day 14 for the 0, 14 procedure and approximately 50% seroconversion with GMTs of 342 and 380 at day 28 (Fig. 2a). Further anti-S antibody detection for the medium and high doses in the 0, 28 procedure showed seroconversion rates of 89% and 93%, respectively, with GMTs of 937 and 929, respectively, at day 28 after immunization (Fig. 2b); anti-N antibody detection showed seroconversion rates of 68% and 78% with GMTs of 570 and 494, respectively (Fig. 2b). These data suggested that the neutralizing antibody was induced by the vaccine in more than 90% of individuals in this adult population and that the elicited antibody response included anti-S and anti-N antibodies.

Safety monitoring

As one of the indicators in the phase II trial, safety monitoring in this study focused on the clinical adverse reactions that occurred 7 days after each inoculation and 28 days after two immunizations. Observation suggested that with the 0, 14 procedure, adverse reactions occurred in 24%, 27.3% and 17.3% of all individuals in the medium-dose, high-dose and placebo groups, respectively, within 7 days after the first and second injections (Table 2); with the 0, 28 procedure, these values were 26.7%, 19.3% and 12%, respectively (Table 2). Most of these reactions were slight pain, itching and redness at the injection site (Table 2). Induced systemic adverse reactions 7 days after the first and second immunizations, mainly including slight fatigue and fever, were reported in 10%, 13% and 14.7% of individuals in the medium-dose, high-dose and placebo groups, respectively, who received the 0, 14 procedure (Table 2) and in 13.3%, 8% and 9.3% of individuals who received the 0, 28 procedure (Table 2). Overall adverse reaction rates during the 28 days after immunization were 24%, 27.3%, and 17.3% and 27.3%, 19.3%, and 12% in the medium-dose, high-dose and placebo groups, respectively, in the 0, 14 procedure and the 0, 28 procedure, respectively (Table 2). No SAEs related to vaccination were reported.

Discussion

After concerns regarding the safety of our inactivated SARS-CoV-2 vaccine in a phase I trial, this study targeted the immunity elicited by the vaccine through the detection of neutralizing antibodies and ELISA antibodies against not only the S-antigen but also the N-antigen. The use of medium and high doses was based upon the detection of neutralizing antibodies in a phase I trial, in which the GMTs in subjects immunized with low (50 EU), medium (100 EU) and high (150 EU) doses reached 18, 54 and 37, respectively (manuscript in submission). Based on these data, medium and high doses were evaluated in this study in two immunization procedures with intervals of 14 and 28 days. The results indicated that vaccine immunization in this population showed a tendency toward a dose-effect relationship for either seroconversion or GMTs of neutralizing antibodies in the two procedures. However, the fact that the 0, 14 procedure presented a seroconversion rate of 96% with GMTs of 30 and 21 in the high-dose group at days 14 and 28 after immunization, respectively, which were higher than the values of the 0, 28 procedure, suggested that a high dose with the 0, 14 procedure could be a better choice for further phase III trials of this vaccine. This result is also supported by the detection of ELISA antibodies against the S-protein and N protein, in which the high-dose group subjected to the 0, 14 procedure showed higher seroconversion rates and GMTs of anti-S and anti-N antibodies than the medium-dose and placebo groups. These data suggested not only that the immunogenicity of this vaccine induced a neutralizing antibody response in 95% of the adult population aged 18-59 years but also that the vaccine had the capacity to elicit anti-N and anti-S antibodies in the ELISA. Although recent reports of SARS-CoV-2 vaccines have provided much data on the neutralizing antibody response, obtaining an understanding of an integrated immune

response that shows protective efficacy still requires more data on the roles played by antibodies against various viral antigens in antiviral immunity. We still wanted to know whether the roles of cytotoxic T lymphocytes (CTLs) and antibody-dependent cellular cytotoxicity (ADCC), related to the antibodies to S-antigen, N-antigen and other viral antigens, could be involved in controlling and eliminating the virus during infection.

Certainly, safety was still a concern in this study of 750 subjects, and our observations provided further evidence of the safety of the vaccine. No SAEs related to vaccine injection were reported. Some mild local and systemic adverse reactions, similar to those observed in the phase I trial, were reported.

NOTES

Author contributions

Conceived of and designed the experiments: QL, CL, ZX and HC. Performed the experiments: YC, XL, YP, MZ, ZZ, RJ, ZY, MX, QY, JW, JP, HZ, YZhang, LW, YJ, JLei, YZheng, YL, RL, LY, PC, HY, YHZhang, JLi, WC, YZou, YZhu and YZhou. Analyzed the data: QL, CL, LL, ZZ, and YZhang. Contributed reagents/materials/analysis tools: ZH, KM, CH, DL, GJ, DL, XX, SF, CC, HZ, JY, YG and TY. The manuscript was drafted by QL.

Acknowledgments

Conflicts of interest

The sponsor of the study played no role in the study design, data and sample collection, data processing, or report writing. The corresponding author had full access to all the data generated by the study and takes full responsibility for the final submission for publication.

References

1.   Gupta P, Goyal K, Kanta P, Ghosh A, Singh MP. Novel 2019-coronavirus on new year's Eve. Indian J Med Microbiol **2019**; 37(4): 459-77.

2.   Jee Y. WHO International Health Regulations Emergency Committee for the COVID-19 outbreak. Epidemiol Health **2020**; 42: e2020013.

3.   Bulut C, Kato Y. Epidemiology of COVID-19. Turk J Med Sci **2020**; 50(SI-1): 563-70.

4.   Asadi-Pooya AA, Simani L. Central nervous system manifestations of COVID-19: A systematic review. J Neurol Sci **2020**; 413: 116832.

5.   Rego GNA, Nucci MP, Alves AH, et al. Current Clinical Trials Protocols and the Global Effort for Immunization against SARS-CoV-2. Vaccines (Basel) **2020**; 8(3).

6.   Azkur AK, Akdis M, Azkur D, et al. Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. Allergy **2020**; 75(7): 1564-81.

7.   Arvin AM, Fink K, Schmid MA, et al. A perspective on potential antibody-dependent enhancement of SARS-CoV-2. Nature **2020**; 584(7821): 353-63.

Figure legends

Fig. 1 Screening, randomization and inclusion in the phase II clinical trial

The analysis set populations in this trial included the safety analysis set (SS; subjects who received at least one dose of the vaccine), full analysis set (FAS; subjects who met the inclusion/exclusion criteria, participated in randomization, received the vaccination, and had at least 1 result for a postvaccination serum test) and per-protocol set (PPS; subjects who met the inclusion criteria and did not meet the exclusion criteria, received the vaccine and had serum test results as per the protocol).

Fig. 2 Immune response induced in human individuals immunized with the inactivated SARS-CoV-2 vaccine in the 0, 14 and 0, 28 procedures.

a. Neutralizing antibodies and ELISA antibodies (IgG) against S protein and N protein induced by the inactivated vaccine in individuals assigned to the immunization procedure with an interval of 14 days.

b. Neutralizing antibodies and ELISA antibodies (IgG) against S protein and N protein induced by the inactivated vaccine in individuals assigned to the immunization procedure with an interval of 28 days.

Control (Con, 0 EU), medium dose (MD, 100 EU) and high dose (HD, 150 EU). The antibody positive judgment threshold is marked with a dotted line in the figure. *, $0.01 < p < 0.05$; **, $0.001 < p < 0.01$; ***, $p < 0.001$.

Table 1 Characteristics of the Participants in the Phase II Trial at Enrollment[*]

Baseline characteristics	0,14 procedure (N=375)			0,28 procedure (N=375)		
	Medium dose group (n=150)	High dose group (n=150)	Placebo group (n=75)	Medium dose group (n=150)	High dose group (n=150)	Placebo group (n=75)
Age group- no (%)						
18-29 yr	28 (18.7)	31 (20.7)	9 (12.0)	35 (23.3)	33 (22.0)	21 (28.0)
30-44 yr	51 (34.0)	67 (44.6)	36 (48.0)	79 (52.7)	79 (52.7)	34 (45.3)
45-59 yr	71 (47.3)	52 (34.7)	30 (40.0)	36 (24.0)	38 (25.3)	20 (26.7)
Age-yr	41.4±10.84	40.3±10.55	41.8±9.75	37.3±9.82	37.8±9.58	36.9±9.63
Sex-no (%)						
Male	52 (34.7)	56 (37.3)	33 (44.0)	53 (35.3)	62 (41.3)	27 (36.0)
Female	98 (65.3)	94 (62.7)	42 (56.0)	97 (64.7)	88 (58.7)	48 (64.0)
Body-mass index	23.4±3.42	23.8±3.47	23.6±3.39	23.9±3.57	23.7±3.45	23.1±2.88

Ethnicity-no (%)						
Han	108 (72.0)	106 (70.7)	59 (78.7)	108 (72.0)	93 (62.0)	52 (69.3)
Yi	32 (21.3)	27 (18.0)	10 (13.3)	28 (18.7)	34 (22.7)	13 (17.3)
Dai	0 (0.0)	0 (0.0)	1 (1.3)	10 (6.7)	15 (10.0)	4 (5.3)
Zhuang	6 (4.0)	8 (5.3)	3 (4.0)	1 (0.7)	1 (0.7)	1 (1.3)
Other	4 (2.7)	9 (6.0)	2 (2.7)	3 (2.0)	7 (4.7)	5 (6.7)

[*] Plus–minus values are means ±SD.

Table 2 Adverse reactions within 0-28 days after vaccination

Adverse reactions	0,14 procedure (N=375)			0,28 procedure (N=375)		
	Medium dose group (n=150)	High dose group (n=150)	Placebo group (n=75)	Medium dose group (n=150)	High dose group (n=150)	Placebo group (n=75)
	number of participants (%)			*number of participants (%)*		
All Adverse reactions within 0-7 days	36 (24.0)	41 (27.3)	13 (17.3)	40 (26.7)	29 (19.3)	9 (12.0)
Solicited injection site	24 (16.0)	27 (18.0)	3 (4.0)	22 (14.7)	18 (12.0)	1 (1.3)
Pain	23 (15.3)	22 (14.7)	3 (4.0)	21 (14.0)	18 (12.0)	1 (1.3)
Itch	3 (2.0)	7 (4.7)	1 (1.3)	2 (1.3)	0 (0.0)	1 (1.3)
Redness	2 (1.3)	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Swelling	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Solicited systemic adverse	15 (10.0)	20 (13.3)	11 (14.7)	20 (13.3)	12 (8.0)	7 (9.3)
Fatigue	9 (6.0)	10 (6.7)	6 (8.0)	9 (6.0)	4 (2.7)	4 (5.3)
Diarrhoea	1 (0.7)	2 (1.3)	4 (5.3)	4 (2.7)	3 (2.0)	0 (0.0)
Fever	4 (2.7)	4 (2.7)	0 (0.0)	4 (2.7)	4 (2.7)	2 (2.7)
Hypersensitivity/ Urticaria	1 (0.7)	1 (0.7)	2 (2.7)	1 (0.7)	1 (0.7)	0 (0.0)

Cough	2 (1.3)	5 (3.3)	0 (0.0)	2 (1.3)	2 (1.3)	0 (0.0)
Nause	1 (0.7)	0 (0.0)	2 (2.7)	3 (2.0)	1 (0.7)	3 (4.0)
Vomiting	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.7)
Mucosal abnormality	0 (0.0)	0 (0.0)	1 (1.3)	2 (1.3)	0 (0.0)	0 (0.0)
Unsolicited Adverse reactions	4 (2.7)	2 (1.3)	1 (1.3)	11 (7.3)	5 (3.3)	1 (1.3)
Overall Adverse reactions within 0-28 days	36 (24.0)	41 (27.3)	13 (17.3)	41 (27.3)	29 (19.3)	9 (12.0)

No serious adverse events (grade 3) related to vaccination were observed within 28 days

Figure 1

Figure 2

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