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SHORT REPORT

Immunogenicity of inactivated COVID-19 vaccines at different vaccination intervals

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ABSTRACT

To evaluate the immunogenicity of inactivated COVID-19 vaccines administered at different intervals. Subjects who had received two doses of inactivated COVID-19 vaccines at an interval of 21 days or 1–7 months were selected to collect 5 ml of venous blood after the second dose for the detection of specific IgG antibody against SARS-CoV-2 using the chemiluminescent immunoassay. Blood samples were collected from 348 and 174 individuals vaccinated at an interval of 21 days or 1–7 months, respectively. Seropositive rate 2 weeks after two doses of vaccination at 21-days and 1–7 months interval was 95.7% and 97.1%, respectively, with no statistically significant difference. The post-vaccination antibody level was 23.7 with 21-days interval, higher than 14.2 with 1–7 months interval. Among the individuals vaccinated with two doses more than 1-month apart, seropositive rate was 98.5%, 90.0%, 91.7%, and 100% with 1- month (1–2 months, 2 months was not included, the same below), 2- month, 3- month, and 4–7 months of interval, respectively, and no statistically significant difference was observed. Appropriate extension of the vaccination interval between two doses of inactivated COVID-19 vaccine does not affect the production of specific IgG antibodies. The inactivated COVID-19 vaccine should be administered in accordance with the recommended vaccination schedule, and the vaccination interval can be extended appropriately under special circumstances.

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1. Introduction

Inactivated COVID-19 vaccine is a kind of whole virion vaccine, which derived from the new coronavirus SARS-CoV-2 through a series of production steps including virus culture, harvesting, inactivation, concentration, purification and adsorption to aluminum hydroxide. Multiple inactivated COVID-19 vaccines had been proved to be safe and effective in previous studies, and thereafter were approved for emergency use in China and abroad.[1–7] The national *Technical Guidelines for COVID-19 Vaccination (first edition)* recommends that the inactivated COVID-19 vaccine should be administered with two doses at an interval of ≥3 weeks, and the second dose should be completed as early as possible within 8 weeks. After the first dose of vaccination, some recipients have to postpone the second dose due to personal reasons such as illness or supply reasons such as vaccine shortage. The effect of extended vaccination intervals on immunogenicity needs to be evaluated in real-world studies. In this study, venous blood of individuals vaccinated with two doses at intervals of 21 days and more than 1-month was collected for antibody detection, so as to provide scientific evidence for evaluating the immunogenicity of inactivated COVID-19 vaccines administered at different intervals.

2. Methods

2.1. Study design and sample size

This study was carried out based on a 1:2 non-randomized controlled design. Individuals who received two doses of inactivated SARS-CoV-2 vaccine at an interval of more than 29 days were assigned to the study group, i.e., extended interval group; individuals who received two doses at a 21-day interval were assigned to the control group, i.e., normal interval group. The sample size was estimated using formula $n = \frac{\left[\mu_{\frac{\alpha}{2}}\sqrt{2\bar{P}(1-\bar{P})}+\mu_{\beta}\sqrt{P_1(1-P_1)+P_2(1-P_2)}\right]^2}{(P_1-P_2)^2}$. Based on the probability level $\alpha = 0.05$ of type I error, and the probability level $\beta = 0.1$ of type II error, the antibody positive rate P1 and P2 of the extended interval group and normal interval group was 90% and 95%, respectively, and the sample size of the extended interval group was at least 140 individuals. Considering the 30% dropout rate, it was planned to include at least 200 recipients in the extended interval group, and 400 recipients in the normal interval group. This study was approved by the Ethic Committee of Beijing Center for Disease Prevention and Control (2020–28) .

2.2. Study procedure

For the extended interval group, individuals who received two doses of inactivated COVID-19 vaccine at an interval of more than 29 days were screened out through the Beijing Vaccination Information Management System. Among them, the individuals who received two doses at an interval of more than 60 days were all included, and recipients with a vaccination interval of 30–

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60 days which were randomly selected and included into study group. Individuals who received two doses at a normal interval were randomly selected from the immunogenicity surveillance carried out at the same period to constitute the control group. The random number method was adopted for the above-mentioned random selection, and the sample size ratio between the extended and normal interval group was 2:1.

All the subjects were enrolled in the principle of informed, voluntary and free of charge, following the signed informed consent. Follow-up was conducted by vaccination site. Approximate 0.5 ml of venous blood was collected 28 days after the second dose in the extended interval group, and before the first dose and 14 days after the second dose respectively in the normal interval group. The window period of blood collection in both groups was 7 days. The blood samples were detected for the IgG antibody by using the chemiluminescence kit manufactured by the Bioscience (Tianjin) Diagnostic Technology Co., Ltd. The IgG antibody in the serum sample and the components in the reagent form a complex of alkaline phosphatase labeled antibody, IgG antibody, recombinant antigen, and magnetic particle. After the substrate is added, the alkaline phosphatase in complex catalyzes the substrate to emit fluorescence. The relative luminescence unite (RLU) of the complex against the substrate can indirectly reflect the IgG antibody level, and the S/CO value>1 was defined as seropositivity in IgG.

All the testing was conducted in the laboratory of Beijing Center for Disease Prevention and Control. Due to the difference in sample collection time, samples of normal and extended interval group were tested at separate time with two batches of kit respectively. However, the quality control data showed the error between two detection results of a same sample is within 1 times of Standard Deviation (See annexure 1).

2.3. Statistical analysis

Microsoft Excel 2019 was used to sort out the database, and SPSS 17.0 was used for statistical analysis. Enumeration data were analyzed by constituent ratio and 95% confidence interval (CI), and the measurement data were analyzed by $\overline{x} \pm s$ if they conformed to normal distribution, and by median (P25, P75) if not. The t-test or nonparametric test was used to compare the differences in the mean or median between the two groups, and the χ^2 test was used to compare the differences in the rates or constituent ratios between the two groups $\alpha = 0.01$.

3. Results

3.1. Basic information

286 and 425 individuals were enrolled in the extended interval group and the normal interval group, respectively. Of them, 174 and 348 individuals agreed to collect blood after the second dose, respectively. The male to female ratio in the two groups was 1.9:1 (115/59) and 2.3:1 (241/107) respectively, and the difference was not statistically significant (χ^2 = 0.534, P= .465); the age was 37.1 ± 9.8 years old and 39.8 ± 9.2 years old, respectively, and the difference was not statistically significant (t= 3.481, P= .478).

In the extended interval group, the vaccination interval ranged from 36 to 200 days with a median value of 117; 65 recipients (37.4%, mean age[SD] 35.4 ± 10.1 years) had an interval of 1-month, 10 recipients (5.7%, 39.8 ± 8.3) had an interval of 2-months, 36 recipients (20.7%, 37.4 ± 9.9) had an interval of 3-months, 46 recipients (26.4%, 40.8 ± 9.2) had an interval of 4-months, 10 recipients (5.7%, 31.5 ± 6.9) had an interval of 5 months and 7 recipients (4.0%, 30.9 ± 6.4) had an interval of 6–7 months. Among the groups of different extended intervals, statistically significant difference of mean age was observed (F= 3.302, P= .007), while no statistically significant difference of gender ration was observed ($\chi 2$ = 4.378, P= .496) among the above-mentioned groups. The median blood collection time (P25, P75) after the second dose was 30 (29, 32) days.

In the normal interval group, the vaccination interval between two doses in the normal interval group was 21–22 days, and the blood was collected 14 days after the second dose.

3.2. Antibody level

Among the 174 recipients of extended interval group, the seropositive rate was 97.1% (169/174) 28 days after the second dose. Among the 348 recipients of the normal interval group, the negative rate before the vaccination was 98.3% (342/348), and the seropositive rate was 95.7% (333/348) 14 days after the second dose. No statistically significant difference of seropositive rates between groups (χ^2 = 0.650, P= .420). The seropositive rate after two-doses vaccination at the interval of 1-, 2-, 3-, 4-, 5-, and 6–7 months was 98.5%, 90.0%, 91.7%, 100%, 100%, and 100%, respectively, with no statistically significant difference (P= .134) (Table 1).

The median antibody level (P25, P75) after vaccination in the extended interval group and normal interval group was 14.2 (7.4, 31.2) and 23.7 (9.5, 45.3) respectively, with statistically significant difference between groups (P< .001). Whereas, no statistically significant difference of medium antibody level was observed among the recipients vaccinated with various extended intervals (P= .178) (Table 1).

The number of recipients in the extended interval group with the S/Co value <1, 1–24, 25–49, ≥50 was 5 (2.9%), 116 (66.7%), 42 (24.1%) and 11 (6.3%), respectively; the number of recipients in the normal interval group with the S/Co value <1, 1–24, 25–49, ≥50 was 14 (4.0%), 165 (47.4%), 92 (26.5%) and 77 (22.1%), respectively. Statistically significant difference of antibody level distribution was observed (χ^2 = 25.835, P< .001), while no statistically significant difference of S/CO value was observed among the groups of different extended intervals (P= .146) (Table 1).

4. Discussion

Three inactivated COVID-19 vaccines have been granted conditional marketing authorization in China, namely the products of Sinopharm Beijing Institute of Biological Products Co., Ltd. (Beijing Institute), Sinopharm Wuhan Institute of Biological Products Co., Ltd. (Wuhan Institute) and Sinovac Life Sciences Co., Ltd. (Sinovac Life Sciences). Clinical trials of the inactivated COVID-19 vaccine from three manufacturers showed that the neutralizing antibody positive rate could reach more than 90% at 28 days after the second dose following the two-dose

Table 1. Antibody data following administration of two doses of inactivated COVID-19 vaccine at different intervals.

		Seropositive		Antibody level				
Group	No. of subjects	Number	Rate (95%CI) (%)	Median S/Co value (P25, P75)	<1	1–24	25–49	≥50
Normal interval group	348	332	95.7 (93.6,97.8)	23.7 (9.5,45.3)	14(4.0%)	165(47.4%)	92(26.5%)	77(22.1%)
Extended interval group	174	169	97.1 (93.5, 98.8)	14.2 (7.4, 31.2)	5 (2.9%)	116 (66.7%)	42 (24.1%)	11 (6.3%)
P			0.420	<0.001		<0.001		
1–2* months	65	64	98.5 (91.8, 99.7)	13.6 (7.3, 24.6)	1 (1.5%)	49 (75.4%)	14 (21.5%)	1 (1.5%)
2–3 months	10	9	90.0 (59.6, 98.2)	20.1 (6.8, 40.2)	1 (8.3%)	5 (66.7%)	3 (19.4%)	1 (5.6%)
3–4 months	36	33	91.7 (78.2, 97.1)	12.3 (4.9, 29.3)	3 (8.3%)	24 (66.7%)	7 (19.4%)	2 (5.6%)
4–5 months	46	46	100 (92.3, 100)	18.1 (7.4, 32.3)	0	28 (60.9%)	16 (34.8%)	2 (4.3%)
5–6 months	10	10	100 (72.3, 100)	22.7 (11.3, 62.6)	0	6 (60.0%)	1 (10.0%)	3 (30.0%)
6–7 months	7	7	100 (64.6, 100)	17.7 (13.8, 52.1)	0	4 (57.1%)	1 (14.3%)	2 (28.6%)
P			0.134	0.178		0.146		

Note: * 2 was not included in this range, and the same for other groups.

immunization schedule at an interval of 14 days, 21 days, or 28 days. For the inactivated COVID-19 vaccine developed by Beijing Institute, the neutralizing antibody titer at 28 days after the second dose following the two-dose immunization schedule at intervals of 14 days, 21 days and 28 days was 170, 283 and 218, respectively. There was no statistically significant difference in the antibody level between the 21-day interval and 28-day interval immunization schedule, which were both higher than the antibody level of two doses at a 14-day interval.[1] For the inactivated COVID-19 vaccine developed by Wuhan Institute, the neutralizing antibody positive rates at 28 days after the second dose following the two-dose immunization schedule at intervals of 14 days and 21 days were both 98%, and the neutralizing antibody titer were 121 and 247, respectively. The antibody level of two doses at an interval of 21 days was higher than that at an interval of 14 days.[2] For the inactivated COVID-19 vaccine developed by Sinovac Life Sciences, the neutralizing antibody positive rate at 28 days after the second dose following the two-dose immunization schedule at intervals of 14 days and 28 days was 94% and 98%, respectively, with no statistical significance. The antibody level of two doses at a 28-day interval was higher than that at a 14-day interval.[3] Therefore, two-dose vaccination at an interval of 14–28 days could all give good antibody positive rate, and an appropriate extension of the interval could improve the antibody level after the full doses vaccination.

Limited by the clinical trial design, the immune effect of long-time intervals can be evaluated only in real-world studies. This study compared the immunogenicity of two doses of inactivated COVID-19 vaccines given at a 21-day interval and an extended interval. The results showed that a relatively good immune response could be obtained after two-dose vaccination within the interval of 21 days to 7 months, with a seropositive rate over 90%, and no downward trend was demonstrated as the dose interval increased. These findings suggested that as the antibody produced, immune memory cells may be also generated after first dose vaccination, which can facilitate a rapid antibody elevation after the second dose administered even at a relatively longer interval from the first dose. Comparison of immunization schedules at different intervals for other vaccines also gave similar results. For example, there was no statistically significant difference in seropositive rate between children vaccinated with two doses of varicella vaccine at an interval of 3 months and 6 months,[8] and there was no statistically significant difference in seropositive rate among the newborn vaccinates against Hepatitis B with an interval of 5, 6, and 8 months between the third and the first dose.[9]

This study employed chemiluminescent immunoassay for qualitative detection of the antibody and S/Co value for indirect indicator of the antibody level. The results showed that the antibody level of recipients vaccinated at a 21-interval was statistically significantly higher than that at extended intervals, while no statistically significant difference was observed in groups of different extended intervals, which may due to the limited sample size. Thus, more evidences from a larger sample size as well as relationship between immune persistence and immune antibody level are needed to be obtained in further studies.

The study indicates that different vaccination intervals of two-doses of inactivated COVID-19 vaccine within 21 days to 7 months all give relatively good immune response. However, since only one dose of the inactivated COVID-19 vaccine cannot produce the expected immune effect, a long delay of the second vaccination will increase the risk of infection. Therefore, it is recommended to complete the vaccination of two doses of inactivated COVID-19 vaccine as early as possible in accordance with the national recommended vaccination schedule. If the second vaccination is not completed in time due to personal or supply reasons, the second dose can be given directly, and there is no need to restart the vaccination schedule.

In this study, subjects in the extended interval group postponed the second dose vaccination due to diseases or other reasons, and the immunogenicity evaluation on them was not pre-specified, resulting three main limitations of this study. First, the sample size in subgroups of different extended intervals is limited and to some degree unbalanced, with statistically significant difference of age among groups, which may lead to a certain degree of bias in the results. Second, the blood collection time after vaccination is slightly different between the normal and extended interval groups (14 days versus 28 days after the second dose), which may also have impact on the evaluation. However, this impact was considered as minor, given the existing evidence that antibody seropositive rates detected at 14 and 28 days after the second dose showed no significant difference.[3] Third, no pre-vaccination blood samples were collected for the extended interval group, and the seroconversion rates cannot be calculated as a result. However, data from the normal interval group showed a seropositive rate of merely 1.7%. Moreover, a large-scale serological survey conducted by China CDC one month after the successful containment of the first wave epidemic reported

a seropositive rate of less than 1% outside the Wuhan City in mainland China.[10] Thus, the seropositive rate was adopted as the endpoint for the immunogenicity evaluation.

5. Conclusion

Appropriate extension of the vaccination interval between two doses of inactivated COVID-19 vaccine does not affect the production of specific antibodies. The inactivated COVID-19 vaccine should be administered in accordance with the recommended vaccination schedule, and the vaccination interval can be extended appropriately under special circumstances.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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