

EFFECT OF PREDATED VACCINATION AND CHINESE HERBAL ADJUVANT RABBIT HEMORRHAGIC DISEASE VACCINE ON IMMUNE RESPONSE IN YOUNG RABBITS

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ABSTRACT

The experiments were conducted to determine effects of precocious vaccination compared to the classical schedule of vaccination and of Chinese herbal adjuvant (CHA) on immune response to vaccine against rabbit hemorrhagic disease (RHD) in young rabbits. In experiment 1, five New Zealand rabbits at 30, 35, 40 or 45 days of age were injected with 3 ml of non-adjuvant inactivated vaccine against RHD, respectively. The results showed that the titer of maternal HI antibody on day 0 in the 35-day-old rabbits was lower than the protective level of 3 log₂; while on days 7 to 49 after the vaccination, the antibody titer was higher than 3 log₂. In experiment 2, thirty New Zealand rabbits at 35 days of age were randomly assigned to 5 treatment groups, representing inoculation with 3 ml of non-adjuvant RHD vaccine, CHA-RHD vaccine, CHA-HA (half dose antigen of RHD vaccine) RHD vaccine, aluminium adjuvant-containing RHD vaccine, or phosphate-buffered saline (PBS), respectively. The results showed that the CHA promoted ($P < 0.05$) the lymphocyte proliferation on day 7 and enhanced serum antibody titer on day 21. These findings from the two experiments suggested that vaccination could be carried out at 35 days and Chinese herbal adjuvant significantly enhanced the immune response to vaccine against RHD in comparison with aluminium adjuvant.

Key words: Rabbit hemorrhagic disease, Chinese herbal adjuvant, Antibody titer, Lymphocyte proliferation.

INTRODUCTION

Rabbit hemorrhagic disease (RHD) is a fatal disease of rabbits caused by RHD virus. In 1984 at the initial epidemic stages, the virus mainly infected adult rabbits at 3 to 4 months of age (Du *et al.*, 1991; Wang *et al.*, 1988). In China, the first vaccination of the vaccine against RHD is usually at 45 days of age, and secondly at 65 days of age (Wang, 2000). However, in recent years, the susceptible rabbits become younger. Even rabbits younger than one month old could be infected by RHD (Shien *et al.*, 2000; Cooke, 2002; Mikami *et al.*, 1999), thus, a predated vaccination must be taken to avoid early infection. On the basis of our recent findings of Chinese herbal adjuvant (CHA), we hypothesized that vaccination date could be predated and CHA could enhance immune response to RHD vaccine in young rabbits. This hypothesis was tested in the present study by determining the dynamic changes of serum HI antibody titers after inoculation with the vaccine against RHD in 30-, 35-, 40- or 45-day-old rabbits, and then the dynamic changes of peripheral lymphocyte proliferation and serum HI antibody titers after injection with the RHD vaccine, or mixture of RHD vaccine and CHA or aluminum adjuvant (AA) in 35-day-old rabbits. The main purpose of these experiments is to find an appropriate vaccinating program to protect the young rabbits from the attack of RHD virus.

MATERIALS AND METHODS

Vaccine Preparation

Inactivated vaccine against RHD (No. 20051201) and AA were supplied by Jiangsu Academy of Agriculture Science. CHA was supplied by Institute of Traditional Chinese Veterinary Medicine of Nanjing Agricultural University, containing astragalus polysaccharides and ginsenosides, the total content of which was 20 mg/ml. CHA-RHD vaccine, AA-RHD vaccine and non-adjuvant RHD vaccine were prepared by mixing 20 ml of the inactivated vaccine with 10 ml CHA, 3 ml AA and 7 ml phosphate-buffered saline (PBS), and 10 ml PBS, respectively, in which the antigen contents were the same. Another CHA-vaccine, containing half dose of antigen named CHA-HA vaccine, was made of 10 ml inactivated vaccine, 10 ml CHA and 10 ml PBS.

Reagents

RPMI-1640 (GIBCO) supplemented with 100 IU/ml benzylpenicillin, 100 IU/ml streptomycin and 10% fetal bovine serum (FBS) was used. Phytohemagglutinin (PHA, Sigma, No. 051201), as a T-cell mitogen, was dissolved into 0.10 mg/ml with RPMI-1640. Lipopolysaccharide (LPS, Sigma, No. 051201), as a B-cell mitogen, was dissolved into 0.025 mg/ml with RPMI-1640. The 3-(4,5-dimethylthiazol-2-yl)-2, 5 diphenyltetrazolium bromide (MTT, Amresco Co.) was dissolved into 5 mg/ml with calcium and magnesium-free (CMF) PBS, pH 7.4. These reagents were filtered through a 0.22 µm syringe filter. PHA and LPS solution were stored at -20°C, MTT solution at 4°C in dark bottles. Dimethyl sulfoxide (DMSO, No. 050601) was produced by Kemiou Institute of Chemical Engineering in Tianjin. Lymphocytes Separation Medium (No. 060218) was the product of Hua-jing Biostix Shanghai Inc. Positive serum and negative serum for RHD vaccine were supplied by Jiangsu Academy of Agriculture Science. Human O erythrocyte was provided by Centre Hospital of Nanjing Military Area.

Animals

New Zealand rabbits at 30 to 45 days of age without any vaccination were housed in wire cages (60×100 cm²) in air-conditioned room at 25°C, and fed with the commercial starter diet. The rabbits and diet were provided by Jiangsu Academy of Agriculture Science.

Experimental design

Five rabbits of each group at 30, 35, 40 or 45 days of age were used in experiment 1, respectively. All the rabbits were inoculated subcutaneously with 3 ml of the non-adjuvant vaccine on neck. On days 0 (before inoculation), 7, 14, 21, 28, 35, 42 and 49 after initiation of the injection, 0.5 ml of blood was sampled from each rabbit for determination of serum HI antibody titers by micro-method (Zhuang *et al.*, 1995). A total of thirty rabbits at 35 days of age were randomly assigned to 5 treatment groups in experiment 2, representing subcutaneous inoculation with 3 ml of the non-adjuvant RHD, CHA-RHD, CHA-HA RHD, AA-RHD vaccines, or PBS, respectively. On days 0 (before inoculation), 7, 14, 21, 35 and 49 after initiation of the inoculation, 0.5 ml blood per rabbit was sampled to determine the serum HI antibody titers. Also, four rabbits from each group were randomly sampled (2 ml blood per rabbit) for assay of T and B lymphocyte proliferation.

Serum HI antibody Assay

Blood samples were drawn from the auricular vein of rabbits and sera stored at -20°C until analysis for serum HI antibody. The analysis was done following the procedure in Zhuang *et al.* (1995). The geometric mean titer was expressed as reciprocal log₂ values of the highest dilution that displayed HI (Zhuang *et al.*, 1995).

Peripheral lymphocyte proliferation assay

Using the procedure described in Wu *et al.* (1993) the blood samples were collected and light absorbance was measured with microliter enzyme-linked immunosorbent assay reader (Model DG-3022, East China Vacuum Tube Manufacturer) at a wave length of 570 nm.

Statistical analysis

Data are expressed as the mean \pm S.D. Duncan's multiple range test was used to analyze the differences among the treatment groups. A P-value of less than 0.05 was taken to indicate statistical significance.

RESULTS

Changes of serum HI antibody titer in rabbits vaccinated at different ages

Dynamic changes of serum HI antibody titer were showed in Figure 1. The maternal antibody titer dropped with the increase of age. In the 30-day-old rabbits, the titers were higher than 3 log₂, while in the 35-day-old rabbits, lower than 3 log₂. On days 7 to 14 after initiation of the vaccination, the antibody titers in the 30-day-old rabbits were higher than 3 log₂, while lower than 1 log₂ on day 49. In the 35-, 40- and 45-day-old rabbits, the antibody titers increased gradually from day 7 after initiation of the vaccination, reached peak-value on day 28, and still higher than 3 log₂ on day 49, which were higher ($P < 0.05$) compared with the 30-day-old rabbits.

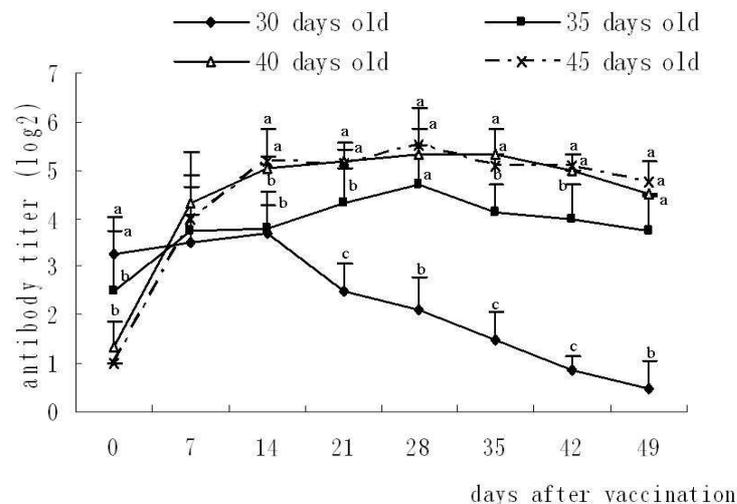


Figure 1: Dynamic changes of serum HI antibody titers in rabbits vaccinated at different age (n=5). Data at the same time point with different letters (a-c) differ significantly ($P < 0.05$)

Changes of serum HI antibody titer in rabbits vaccinated with different vaccines

The dynamic changes of serum HI antibody titer in rabbits vaccinated with different vaccines against HRD were listed in Table 1. On day 0 after initiation of the vaccination, the antibody titers were not significant different ($P > 0.05$) among all of the five treatment groups. The titers in the three adjuvant vaccine groups were higher on days 7 ($P > 0.05$) and 14 ($P < 0.05$) compared with the non-adjuvant vaccine group. On days 21, 35 and 49, the titers in the CHA vaccine group were higher ($P < 0.05$) in comparison with the other four groups. On days 14 and 35, the titers in the CHA-HA vaccine group were higher ($P < 0.05$) than that of the non-adjuvant groups. At all time points, there were no significant differences ($P > 0.05$) between the CHA-HA vaccine group and AA-vaccine group, while the titers in the four vaccinated rabbits were higher ($P < 0.05$) compared with the PBS-inoculated rabbits.

Table 1: The dynamic changes of serum HI antibody titers in rabbits vaccinated with different vaccines against rabbit hemorrhagic disease (log₂; n=6)

Treatments	Day after initiation of the vaccination					
	0	7	14	21	35	49
Non-adjuvant vaccine	2.50±2.00	3.00±1.29 ^a	3.50±0.82 ^b	4.00±0.41 ^b	3.80±0.58 ^c	3.50±0.82 ^b
CHA vaccine	2.67±1.15	3.75±0.96 ^a	4.50±1.29 ^a	6.00±0.82 ^a	6.25±0.96 ^a	6.00±0.82 ^a
CHA-HA vaccine	2.33±1.53	3.25±0.50 ^a	4.25±0.50 ^a	4.50±0.41 ^b	4.50±0.58 ^b	4.00±0.82 ^b
AA vaccine	2.33±0.58	3.25±1.26 ^a	4.50±1.29 ^a	4.50±0.58 ^b	5.00±0.82 ^b	3.13±0.25 ^b
PBS	2.30±1.00	1.00±0.82 ^b	0.67±0.47 ^c	0.25±0.28 ^c	0.33±0.47 ^c	0.25±0.50 ^c

^{a-c} Means ± S.D. within a column with different superscripts differ significantly (P<0.05)

AA, aluminium adjuvant; CHA, Chinese herbal adjuvant; HA, half dose of antigen; PBS

Table 2: The dynamic changes of peripheral T lymphocyte proliferation in rabbits vaccinated with different vaccines against rabbit hemorrhagic disease (OD₅₇₀; n=4)

Treatments	Day after initiation of the vaccination					
	0	7	14	21	35	49
Non-adjuvant vaccine	0.268±0.017	0.282±0.017 ^b	0.304±0.086	0.304±0.046	0.303±0.089	0.306±0.068
CHA vaccine	0.258±0.043	0.378±0.049 ^a	0.331±0.083	0.325±0.037	0.323±0.069	0.333±0.037
CHA-HA vaccine	0.283±0.063	0.354±0.044 ^a	0.334±0.038	0.324±0.037	0.318±0.088	0.320±0.046
AA vaccine	0.268±0.071	0.375±0.068 ^a	0.341±0.097	0.331±0.088	0.327±0.010	0.325±0.037
PBS	0.292±0.033	0.280±0.034 ^b	0.282±0.026	0.285±0.031	0.288±0.075	0.300±0.079

^{a-b} Means ± S. D. within a column with different superscripts differ significantly (P<0.05). AA, aluminium adjuvant; CHA, Chinese herbal adjuvant; HA, half dose of antigen; PBS

Table 3: The dynamic changes of peripheral B lymphocyte proliferation in rabbits vaccinated with different vaccines against rabbit hemorrhagic disease (OD₅₇₀; n=4)

Treatments	Day after initiation of the vaccination					
	0	7	14	21	35	49
Non-adjuvant vaccine	0.234±0.067	0.301±0.081 ^{bc}	0.375±0.052 ^b	0.322±0.042 ^b	0.306±0.091 ^{ab}	0.306±0.054 ^{ab}
CHA vaccine	0.206±0.062	0.430±0.036 ^a	0.444±0.042 ^a	0.390±0.037 ^a	0.348±0.099 ^a	0.339±0.064 ^a
CHA-HA vaccine	0.222±0.084	0.308±0.094 ^{bc}	0.362±0.055 ^b	0.346±0.037 ^b	0.300±0.088 ^{ab}	0.299±0.053 ^{ab}
AA vaccine	0.232±0.062	0.342±0.073 ^b	0.390±0.020 ^b	0.340±0.035 ^b	0.304±0.097 ^{ab}	0.292±0.051 ^{ab}
PBS	0.248±0.124	0.258±0.019 ^c	0.260±0.012 ^c	0.270±0.019 ^c	0.280±0.075 ^b	0.274±0.057 ^b

^{a-c} Means ± S. D. within a column with different superscripts differ significantly (P<0.05). AA, aluminium adjuvant; CHA, Chinese herbal adjuvant; HA, half dose of antigen; PBS

Changes of peripheral T and B lymphocyte proliferation in rabbits vaccinated with different vaccines

The dynamic changes of peripheral T lymphocyte proliferation (Table 2) showed that on day 0 after initiation of the vaccination, the OD₅₇₀ values were not significant different (P>0.05) among all of the five treatment groups; on day 7, the values in the three adjuvant vaccine groups were higher (P<0.05) compared with the other two groups, and there were no significant differences (P<0.05) among the three adjuvant vaccine groups. The dynamic changes of peripheral B lymphocyte proliferation (Table 3) showed that on day 0 after initiation of the vaccination, there were no significant differences (P<0.05) among the OD₅₇₀ values in all of the five treatment groups; the values of CHA vaccine group were higher (P<0.05) compared with the other four groups on days 7, 14 and 21. On days 35 and 49, the values of the CHA vaccine group were higher in comparison with the other three vaccinated groups (P>0.05) and PBS-inoculated groups (P<0.05).

DISCUSSION

The serum antibody titer is an indicator of humoral immunity. In our previous study, we found that serum antibody titer higher than 3 log₂ could provide 100% protection for rabbits from rabbit hemorrhagic disease, 2 log₂, only 50%, lower than 2 log₂, a little protection. Therefore, 3-4 log₂ of antibody titer was called protective antibody titer. The results from the experiment 1 showed that the maternal antibody titers in the 35- to 45-day-old rabbits were lower than the protective titer (3 log₂), which were difficult to resist the infection of RHD virus and needed vaccinating. Thus, when the

inoculation age was predated to 35 days old, the antibody titer increased rapidly, and was higher than 3 log₂ for 7 weeks and 4 log₂ for 3 weeks after vaccination. In the experiment 2, antibody titers of the three adjuvant vaccine groups were significantly higher compared with the non-adjuvant vaccine group on day 14 after the vaccination. On days 21, 35 and 49, the titers of the CHA vaccine groups were significantly higher in comparison with the non-adjuvant vaccine groups and AA vaccine groups, which suggested that the immune enhancement of CHA was better than AA. Collectively, these findings suggested that the first vaccination of young rabbits should be predated to 35 days of age in order to resist the infection of RHD virus, and the CHA adjuvant could significantly enhance the production of HI antibody against RHD vaccine in the young rabbits. Lymphocytes proliferation is the indicator reflecting the state of cellular immunity in animal body. Our results showed that on day 7 after the vaccination, peripheral T lymphocyte proliferation significantly increased in the CHA- and AA-vaccine groups compared with the other groups. On days 7, 14 and 21 after the vaccination, the proliferation of B lymphocyte in the CHA groups were significantly higher compared with the AA groups, which suggested that the CHA possessed better effect on enhancing the cellular immunity in comparison with the AA, in addition with the low side-effect and resourceful advantages of the CHA, we suggested that the classical adjuvant AA could be replaced by the new adjuvant CHA.

CONCLUSIONS

In summary, our findings in the present study suggested that the first vaccination in the young rabbits could be predated to 35 day old. Combination of vaccine with CHA could enhance immune response against the RHD in the animals. Nevertheless, comparison of the CHA adjuvant with other commercial “classical” vaccine (with oil adjuvant for example) should be realized to confirm if the CHA adjuvant could replace the “classical” adjuvant.

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