A COMPARISON OF EFFECT OF TRYPANOSOMA EVANSI ADJUVANT VACCINES*

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Introduction

Killed Trypanosoma evansi (T. evansi) treated by phosphate buffered saline (PBS), using weanling mice for the effective immune rest, has been reported previously(4) The effect of this vaccine was found inconstant result of its immune response. This reason is still unknown. For strength or constancy of immunizing effect, it should have considered to use adjuvants(1,2,3,) in the vaccines.

Although zeolite is a very powerful absorbent material but it has not applied in the production of biological products of Trypanosoma. This study describes the comparison of the effect of vaccines which were treated by PBS, zeolite, complete Freund's. ADE, potassium aluminum sulfat (PAS), and freezethawing(1,5) vaccines.

Materials and methods

Trypanosoma strains-- Four strains, designed as D3, D4, D5 and D6 shown as previous report(4) isolated from shepherd dogs, were used in this experiment. Blood from heart of moribund mouse, when these strains were inoculated into mice, contained 8-10 x $10^5/mm^3$ or T. evansi.

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Mice -- Fifteen to 25 grams body weight of DD strain weanling mice bred in this laboratory were used in this experiment.

Zeolite (Zl) -- The powder of natural zeolite which applied in soil improvement was used in this experiment. The chemical composition of dry material treated at 105°C is as follows: SiO_2 72,97%, Al_2O_3 12.35%, F Fe_2O_3 3.39%, TiO_2 0.06%, CaO 0.04%, MgO 1.18%, K_2O 0.81% and Na_2O 0.76%.

Methods of vaccine preparations :

BBS vaccine (PBSV) -- Vaccine preparation was same as previously(4) After preparation, vaccine was kept at 4°C refrigerator for 72 hours before use.

Zeolite vaccine (ZIV) -- One gram of zeolite powder was mixed with 100 ml of PBS in the 200 ml flask and aut claved. After cooling, supernatant layer without filtration was used as PBS vaccine method. Zeolite suspension was mixed with moribound mouse heart blood and adjusted final concentration to 8-19 x 10^6/ml of T. evansi.

Complete Freund's adjuvant vaccine (CFAV) -- Equal volume of CFA was mixed with PBS which contained 1.6 - 2 x 10^7/ml of T. evansi.

Potassium aluminum sulfate vaccine (PASV) -- Ten percent of PAS was added to PBS which contained 8-10 x 10^6/ml of T. evansi. After 3 hours standing and absorption, the supernatant layer was discarded and changed equal volume of normal saline. Then they were kept at refrigerator before use.

Freeze-thawing vaccine — Some of PBSV and ZIV were made 3 times of freeze-thawing treatment.

ADE vaccine (ADEV) -- Equal volume of commercial ADE and PBSV were mixed.

Vaccination : One-tenth ml of each PBS, Zl, ADE, freeze-thawing and PAS vaccines was inoculated subcutaneously into back area once or twice and 0.05 ml of DFA vaccine by intra-pad.

Challenge : Challenge tests were performed on the third week after vaccination. The challenged number pf T. evansi was 40-50. The effect of vaccine was determined between two weeks after challenge according to their survival or death of mouse numbers.

Results

The results of the effectiveness of once or twice immunization with 4 strain of PBS and Z1 vaccines, and challenge with homologous strain were shown as Table 1. The whole survived mice after challenge were 9 out of 34 (26.5%) and 25 out of (78.1%) of once and twice injection in PBSV respectively. There fore, the survived mice of ZIV were 28 out of 40 (70.0%) and 35 out of 40 (87.5%) of once and twice injection respectively. However, twice injections showed higher protection than the once in both vaccines.

The results in Table 2 are twice injections with D3 strain of PBS and Z1 vaccines and using 4 different strains for challenge. The total survived mice were 31 out of 40 (77.5%) and 36 out of 40 (90.0%) in PBS and Z1 vaccines respectively.

Table 3 shows a comparison of the effectiveness of different immune routes with 4 strain of CFAV, and challenge with homo- and heterologous strain. The results were 38 out of 40 (95.0%), 39 out of 40 (97.5%), 13 out of 20 (65.0%) and 25 out of 40 (62.5%) survived mice in D3, D4, D5 and D6 vaccine strains respentively. From this experiment, there was no difference in their survivers by intra-pad of 2, 3 or 4 feet and subcutaneous injection when challenge strains were used homologous (D3, D4), but the protection of D6 immune strain was lower that the homologous when using heterologous (i.e., D3 and D4 strain) for challenge strains. The whole survived mice were 26 out of 40 (65.0%) in ADEV (Table 4).

Table 5 shows the results of effectiveness of PAS and freeze-thawing treated PBS and ZL vaccines. The survived mice were 16 out of 40 (40.0%) in PASV and freeze-thawing treated PBS and Z1 vaccines were 20 out of 40 (25.0%) and 20 out of 40 (50.0%) respectively. From this results, the survived mice were freeze-thawing vaccine was lower than non-treated vaccine (Tables 1 & 2).

From above results of survived mice the difference was found between the Trypanosoma strains of various vaccines, but it was hard to find their general difference between the seven vaccines.

Discussion

In this experiment, seven difference T. evansi vaccines, such as

PBS, Zl, CFA, ADE, PAS and freeze-thawing of PBS and Zl, were used for the comparisen of their immune effectiveness in the mouse. The effective vaccines were determined according to the number of survived mice after challenge. The order of the effectiveness were : CFA, Zl, PBS, ADE, freeze-thawing, PAS, and freeze-thawing PBS vaccines.

The effect of the PBSV will increase when zeolite is added to the PBS. It is more safe if vaccine is preserved at refrigerator over five days.

From economic observation, although CFAV is the best effect of all seven vaccines, but it is hard to recommend with the exception of canine to use in other domestic animals. Morever, it is hard absoption hence it is difficult to apply in human. Therefore, the production of immune antiserum thought to be the only best useful.

Trypanosoma vaccine shows higher effect in survived mice when challenge strain is using homologous vaccine production. High survivors also show in twice vaccination of PBS and Zl vaccines when challenge strain is using heterologous, whereas the result of CFAV showed lower in once injection.

PBS and Zl of freeze-thawing vaccines showed lower effectiveness of protection. This result is different from the predecessor's report, (1, 5) but the reason is unknown.

Mouse is the most sensitive animal to T. evansi. Vaccine could be recomended to use in domestic animals when its effectiveness was proved. Whereas, it it was applied in human, it should remove serum protein.

Summary.

Trypanosoma evansi infected mouse blood was used for production of seven vaccines --- PBS, zeolite, complete Freund's adjuvant, ADE adjuvant, potassium aluminum sulfate, freeze-thawing PBS and freeze-thawing zeolite vaccines were used for the comparison of their immune effectiveness in the mouse. The order of the effectiveness were complete Freund's adjuvant, zeolite, PBS, ADR adjuvant, freeze-thawing zeolite, potassium aluminum sulfate and freeze-thawing PBS vaccines.

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However, twice injections showed higher protection than the once in both PBS and zeolite vaccines, but the effect was decreased when both vaccines were made freeze-thawing treatment.

From four strain (D3, D4, D5, and D6) of Trypanosoma, it was found the strain D5 did not suit to use in vaccine production.

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Table 1. Comparison of effectiveness by once or twice injection with 4 strain of PBS and zeolite vaccines and use homologous strain for challenge.

Vaccine	Time of Injection	Type of Strain	No. of Injected	No. of Challenged	No. of Survived	% of Survived
PBS	1	D3	10	10	4	9/34 (26.5%)
		D4	10	4	2	
		D5	10	10	0	
		D6	10	10	3	
	2	D3	10	10	9	25/32 (78.1%)
		D4	10	2	2	
		D5	10	10	6	
		D6	10	10	8	
Zeolite	1	D3	10	10	8	28/40 (70.0%)
		D4	10	10	9	
		D5	10	10	4	
		D6	10	10	7	
	2	D3	10	10	10	35/40 (87.5%)
		D4	10	10	10	
		D5	10	10	7	
		D6	10	10	8	
Control		D3		10	0	0/40 (0%)
		D4		10	0	
		D5		10	0	
		D6		10	0	

* the body weight of used mice was 15-25 gm.

Tabel 2. Comparison of effectiveness by twice injections with D3 strain of PBS and zeolite vaccines and use 4 homo or heterologous strains for challenge

Vaccine	Challenged strain	No.of. Injected	No. of Challenged	No. of Survived	% of Survived
PBS	D3	10	10	8	31/40 (77.5%)
	D4	10	10	7	
	D5	10	10	8	
	D6	10	10	8	
Zeolite	D3	10	10	10	36/40 (90.0%)
	D4	10	10	8	
	D5	10	10	9	
	D6	10	10	9	
Control			10	0	0%

* The body weight of used mice was 15 gm.

Table 3. Comparison of effectiveness of different immune place with 4 strain of complete Freund's adjuvant vaccine and use homo - or heterologous strains for challenge.

Vaccine Strain	Challenge Strain	No. of Injected	No. of Challenged	Time of Injection	Place of Injected	No. of Survived	% of Survived
D3	D3	10	10	1	2 post-feet	9	28/40 (95.0%)
		10	10	1	3 feet	10	
		10	10	1	4 feet	10	
		10	10	1	SC	9	
D4	D4	10	10	2	once in 2 post-feet, once in Sc		39/40 (97.5%)
		10	10	2	once in 3 feet, once in Sc	10	
		10	10	2	once in 4 feet, once in Sc	10	
		10	10	2	SC	10	
D5	D5	10	10	1	SC	6	13/20 (69.0%)
		10	10	2	SC	7	
	D3	10	10	1	SC	5	25/40 (62.5%)
	D4	10	10	1	SC	6	
	D5	10	10	1	SC	6	
	D6	10	10	1	SC	8	
Control		Same of Table 2					

* Thu body weight of used mice was 15-25 gm.

Table 4. Effect of immune response with 4 strain of ADE adjuvant vaccine.

Vaccine Strain	No. of Injected	No. of Challenged	No. of Survived	% of Survived
D3	10	10	6	
D4	10	10	7	26/40 (65.0%)
D5	10	10	7	
D6	10	10	6	

* The body weight of used mice was 15 gm.

Table 5. Effect of potassium aluminum sulfate and freeze-thawing treated PBS and zeolite vaccines.

Vaccine	Vaccine Strain	Challenge Strain	No. of Injected	No. of Challenged	No of Survived	% of Survived
PAS	D3	D3	10	10	3	16/40 (40.0%)
	D4	D4	10	10	4	
	D5	D5	10	10	5	
	D6	D6	10	10	4	
Freeze-thawing Zeolite	D3	D3	10	10	2	10/40 (25.0%)
	D4	D4	10	10	3	
	D5	D5	10	10	2	
	D6	D6	10	10	3	
Freeze-thawing Zeolite	D3	D3	10	10	4	20/40 (50.0%)
	D4	D4	10	10	8	
	D5	D5	10	10	4	
	D6	D6	10	10	4	
Control		D3		10	0	0/40 (0%)
		D4		10	0	
		D5		10	0	
		D6		10	0	

* The body weight of used mice was 15-18 gm.