

CALCIUM PHOSPHATE AS AN ADJUVANT FOR INACTIVATED RIFT VALLEY FEVER VACCINE

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Abstract

Vaccination to protect animal against infectious diseases may be enhanced by using adjuvants that can selectively stimulate immunoregulatory responses. A novel adjuvant composed of calcium phosphate (CAP) was compared with the commonly used aluminium hydroxide gel adjuvant for its ability to induce immunity to Rift Valley Fever virus. Results indicated that CAP was more potent as an adjuvant than alum, induced high titres of antibody. Furthermore, it is economical, simple to manufacture and it is a natural constituent of the animal body.

INTRODUCTION

Rift Valley Fever (RVF) is an arthropod-borne viral disease affecting animals and human. It is an economically important viral disease and widely distributed in different localities of Africa and Asia where periodic epizootic and epidemic occurred causing heavy losses among lambs and calves (Woods *et al.*, 2002 and Fagbo, 2002).

RVF disease is caused by RNA single stranded virus belonging to family Bunyaviridae (WHO, 1982 and Connie, 1996). The appearance of RVF disease in Egypt in 1977 (Imam *et al.*, 1977), and its reappearance in 1993 (El-Gabery *et al.*, 1994) increased the demand to develop a potent inactivated RVF vaccine. The adjuvants are modulators of the immune system and their function is to improve vaccine efficacy in order to offer protection against infection. One of these adjuvants is calcium phosphate which is a novel adjuvant elicited little or no inflammation at the site of inoculation and induced higher titre of neutralizing antibody (Bio Sante Pharmaceuticals Inc., 2000). Alum compounds are the most extensively used adjuvants in licensed vaccines. Although they effectively enhance immune response, they cause severe inflammatory reaction at the injection site and the duration of this inflammation is somewhat long as cited by Goto *et al.* (1997) who reported that the local tissue reactions caused by injection of CAP gel completely ceased by the 4th week, while, irritation caused by aluminium hydroxide gel persisted for 8 weeks. So, the aim of this work is to study the effect of CAP (as an adjuvant) when added to Rift Valley Fever binary inactivated virus on the immune response of vaccinated sheep.

MATERIALS AND METHODS

1. Animals

1.1. Mice (Swiss albino mice)

1.1.1. Adult mice

Twenty- one- twenty- eight days old mice were used for toxicity and potency test for both calcium phosphate and vaccines, respectively.

1.1.2. Baby mice

three -five days old mice were used for safety of the prepared inactivated virus.

1.2. Sheep

1.2.1. Fourteen susceptible balady sheep six month age were used for the potency test of the vaccines.

1.2.2. Eight lambs of 5-10 days old were used for safety of the RVF vaccine with different calcium phosphate concentrations.

2. Virus

RVF virus ZH-501 with a titre of $7.5 \log_{10} \text{TCID}_{50}/\text{ml}$ were kindly supplied by RVF Department, Abbasia, Cairo. It was isolated from human patient in Zagazig, Sharquia Province during outbreak in 1977.

3. Conjugate

Horseradish peroxidase conjugate labeled antispecies (antisheep) was purchased from Sigma Company. It was diluted in PBS immediately before use for ELISA test.

4. Adjuvant

1.1.1. Aluminium hydroxide gel:

2% gel was purchased from Honil Limited, London, United Kingdom.

1.2.1. Calcium phosphate:

Composed of:

1. Calcium chloride (Winlab).
2. Dibasic sodium phosphate (El-Nasr Pharmaceutical Chemicals Co.).
3. Sodium citrate (Analar).

It was prepared according to Bio Sante Pharmaceuticals, Inc., Smyrna, Georgia (2000).

Toxicity test

Adult mice were used for the toxicity of CAP adjuvant in vaccine preparation. Three groups of mice (15 per each) one inoculated I/P and the second S/C while the third group was kept as a control, and all groups were observed for 10 days post- inoculation.

Preparation of the vaccine

1. Virus

RVF ZH-501 was inactivated by binary ethyleneimine according to Eman (1995), then different forms of vaccines were prepared, one with 25% aluminium hydroxide gel

and the three others with (75%, 50% and 25%) calcium phosphate, respectively.

2. Addition of CAP adjuvant

CAP was added with different concentrations to the inactivated virus as (75%, 50% and 25%).

Evaluation of the vaccine

1. Sterility test

It was done according to OIE (2000).

2. Safety test

It was performed according to El-Nimr (1980) and Eman (1998).

a. Baby mice

They were inoculated I/C.

b. Lambs

Nine lambs were inoculated each with 10ml of the vaccine (5ml I/P and 5ml S/C) then, these animals were observed for 10 days for any signs of RVF disease or death (El-Nimr, 1980 and Eman, 1995).

Potency test

Adult mice were inoculated I/P by two doses of the vaccine one week apart, and then, challenged to calculate the ED₅₀ for each formula of the vaccine separately according to **Randall *et al.* (1964)**.

Experimental Design

Fourteen balady sheep were divided into 5 groups:

Group (1) Three sheep were vaccinated S/C with inactivated RVF vaccine with 75% CAP.

Group (2): Three sheep were vaccinated S/C with inactivated RVF vaccine with 50% CAP.

Group (3): Three sheep were vaccinated S/C with inactivated RVF vaccine with 25% CAP.

Group (4): Three sheep were vaccinated S/C with inactivated RVF vaccine with aluminium hydroxide gel (commercial one).

Group (5): Two sheep were kept as control non-vaccinated.

All animals were observed for 6 months post inoculation for sero-conversion.

Serological tests

1. Serum neutralization test

It was done according to (Walker, 1975).

2. Indirect enzyme linked immunosorbent assay (indirect ELISA)

It was done according to Voller *et al.* (1976).

RESULTS

Table 1. Results of toxicity test in mice

Adjuvant	Mice		Control
	S/C	I/P	
CAP	0/15*	0/15	0/15

* Number of dead mice over number of survived mice.

Table 2. Results of sterility, safety and potency test of the prepared vaccine.

Type of vaccine	Sterility	Safety		Potency ED ₅₀ /ml
		Baby mice*	Lamb**	
CAP 75%	Sterile	0/8	0/2	0.0006/ml
CAP 50%	Sterile	0/8	0/2	0.0003/ml
CAP 25%	Sterile	0/8	0/2	0.0005/ml
Aluminium hydroxide gel	Sterile	0/8	0/2	0.0006/ml

The minimum permissible limit of ED₅₀/ml is 0.02.ml.

* Safety test in baby mice performed of inactivated RVF virus without adjuvant.

** Safety in lamb = No thermal or clinical reaction or manifestation.

DISCUSSION

Rift Valley fever is one of the serious viral zoonotic diseases in Egypt. It is an acute arthropod-borne viral disease causing high mortality and morbidity among sheep, cattle and goats (Castro and Heuschele, 1992). Many studies were performed to improve RVF vaccines and to increase their efficiency and duration of immune response. Many adjuvants have routinely been used for research and in veterinary vaccines. However, toxicity and physicochemical properties that affect their manufacture have limited their use in vaccines. A new formulation of calcium phosphate was used as an adjuvant. It is easy to manufacture on industrial scale showing less variation in quality and physicochemical properties as well as in pH between batches than alum (Feldkamp *et al.*, 1982; Gateff *et al.*, 1973 and Kreuter *et al.*, 1978).

Nowadays, studies are directed to choose an adjuvant which can induce a high and long lasting immunity. From an immunological point, calcium phosphate enhances the immune response to viral antigen, the level of protection against viral infection, as well as, it is a natural constituent of the animal body (Bio Sante Pharmaceuticals, Inc., Smyrna, Georgia, 2000).

When the toxicity test was carried out on adult mice, the result revealed that neither S/C nor I/P routes of injection elicited inflammation at the site of injection or any signs of toxicity during the test as shown in Table 1. All batches of the prepared vaccines

were sterile and safe when inoculated in baby mice and lambs which showed no variation of body temperature of lambs or no signs of illness and no deaths were observed in mice and lambs. The most potent vaccine was that containing 50% calcium phosphate as an adjuvant as its ED_{50}/ml was 0.0003/ml followed by vaccine containing 25% CAP as an adjuvant as its ED_{50}/ml was 0.0005/ml and the last one was 75% calcium phosphate as its ED_{50}/ml was 0.0006/ml as shown in Table 2. All these batches were within the permissible limit as cited by Randall *et al.* (1964) who said that the ED_{50} must not be more than 0.02/ml. The ED_{50} of aluminium hydroxide gel vaccine, batch was 0.0006/ml. These results agreed with Gehan (1990) and Eman (1995), when they used alum gel vaccine as there is no available data on RVF vaccine adjuvated with calcium phosphate.

The immune response of vaccinated sheep was tested by SNT (Table 3 and Fig. 1) that showed the neutralizing indices of all groups of sheep. It was noticed that sera of sheep vaccinated with RVF vaccine with 50% calcium phosphate gave the highest level at second week (mean of NI equal 1.5) as Pini *et al.* (1973) suggested that the protective titre was 1.5 \log_{10} TCID₅₀/ml and reached its peak at 20th week post- vaccination with a mean of NI (3.8). This agreed with Biosante Pharmaceuticals Inc. (2000) who found that Herp's simplex virus type two (HSV-2) plus calcium phosphate gave high antibody level at 6 weeks after immunization and still was high up to the week fourteen. In case of RVF vaccine with 25% CAP adjuvant, the protective level appeared at 3rd week post vaccination with an average of (1.7 NI), while, RVF vaccine with 75% adjuvant, the protective level appeared at post vaccination with an average of (1.6 NI) and reached its peak at the 16th week post- vaccination being 3.4 NI. With The vaccine of aluminium hydroxide gel, the protective level appeared at 3rd week post- vaccination with an average 1.6 NI and reached its peak at 12th week post- vaccination being 2.6 NI.

The result of SNT was correlated with that obtained by ELISA test as shown in Table 4 and Fig. 2. This agreed with Eman (1995) and Hassan *et al.* (2001), but they used inactivated Rift Valley Fever inactivated aluminium hydroxide gel vaccine.

From the previous data, CAP adjuvant induces no inflammation at site of entry and induces immunological enhancement without toxicity and gives a higher titer of antibody earlier than aluminium hydroxide gel, further more, it is easy to manufacture on an industrial scale and shows insignificant variation in quality and physicochemical properties between batches in production condition.

REFERENCES

1. Bio Sante Pharmaceuticals, Inc., Smyrna, Georgia. 2000. Calcium phosphate nanoparticle adjuvant. *Clinical and Diagnostic Laboratory Immunology*, November, 2000 Vol. 7, No. 6: 899-903.
2. Castro, A. E. and W. P. Heuschele. 1992. Rift Valley Fever. *Vet. Dia. Virol.*, 1992.
3. Connie, S. S. 1996. Bunyaviridae: The viruses and their replication. *Field Virology*. 3rd ed. Vol. 1, Chapter 47. Philadelphia, Lippincott, Raven.
4. El-Gabery, G. H., M. A., Nawal A., Hadia. M. M. Fathia. and N. N. Ayoub. 1994. Unclassical picture of RVF in man and animals in Aswan governorate in May 1993. *Vet. Med. J.*, Giza, 42 (1): 135-138.
5. El-Nimr, M. M. 1980. Studies on the inactivated vaccine against RVF. Thesis, Ph.D. Fac. Vet. Med., Assiut Univ., Egypt.
6. Eman, M. S. 1995. Studies on Rift Valley Fever vaccine inactivated with binary. Ph. D. Vet. Sc., Fac. Vet. Med., Cairo Univ., Egypt.
7. Fagbo, S. F. 2002. The involving transmission pattern of Rift Valley Fever in the Arabian Peninsula. *AMNY Acad. Sci.*, 2002 Oct., 969: 201-204.
8. Feldkamp, J. R., White, J. L. and Hem, S.L. (1982): Effect of surface charge and particle size on gel structure of aluminium hydroxide carbonate gel. *J. Pharm. Sci.*, 71: 43-46.
9. Gateff, C., E. H. Relyveld G. G. and Le. 1973. Etude d'une nouvelle association vaccinal quintuple. *Ann. Microbiol.*, (Inst. Pasteur), 124B: 387-409.
10. Gehan, K. M. 1990. Studies on Rift Valley Fever among animals in Egypt. Thesis Ph.D. Inf. Dis., Fac. Vet. Med., Zagazig Univ., Egypt.
11. Goto, N., H. Kato and J. I. Maeyama. 1997. Local tissue irritating effects and adjuvant activities of calcium phosphate and aluminium hydroxide with different physical properties. *Vaccine*, 15: 1364-1370.
12. Hassan, K. Z., K. A. Elian, and M. M. Taha. 2001. Some studies on sheep vaccinated with Smithburn attenuated Rift Valley Fever vaccine. *Egypt. J. Agric. Res.*, 79 (3).
13. Imam, Z.E.I. M. A. A., Darwish R. El-Karamany and F. Omar. 1977. A preliminary report on an epidemic of Rift Valley Fever in Egypt. *J. Egyptian Public Hlth. Assoc.*, 52: 417-418.
14. Kreuter, J. and I. Haenzel 1978. Mode of action of immunological adjuvants. Some physicochemical factors influencing the effectivity of polyacrylic adjuvants. *Infect. Immun.*, 19: 667-675.

15. OIE. 2000. Rift Valley Fever.
16. Pini, A., L. J. Lund and S. J. Davis. 1973. Fluorescent and neutralizing antibody response to injection by RVF. *J. S. Afr. Z. Med. Ass.*, 44 (11): 161-165.
17. Randall, R., L. N. Binn and V. R. Harrison 1964. Immunization against Rift Valley Fever virus, studies on the immunogenicity of lyophilized formalin inactivated vaccine. *J. Immun.*, 93 (2): 293-299.
18. Voller, A., D. E. Bidwell and A. Bartlett. 1976. Enzyme immunoassay in diagnostic medicine, theory and practice. *Bull. W. H. O.* 53: 55-56.
19. Walker, S. J. 1975. Rift Valley Fever: A review committee on foreign animal diseases, United State of Animal Health Association, US Army Med., Res. Inst. Inf. Dis., Frederick, Maryland.
20. WHO. 1982. Rift Valley Fever and emergent human and animal problem. WHO Offset, Publication No. 63.
21. Woods, C. W., A. M., Karpati T., Greint W. McCarthy P., Galuruku E., Muchiri L., Dunster A., Henderson A. S. Khan R., Swanepoel, I., Bonmarin R., Marlin P., Mann B. L., Smoak M., Ryan T. G. Ksiazek, R. K. Arthur N. D., Kuyeze N. N., Agafa C. Peters and WHO. 2002. Hemorrhagic fever task force. *Emerging infectious Diseases*, 8 (2): Feb., 2002.

إستخدام فوسفات الكالسيوم كمحسن مناعى للقاح حمى الوادى المتصدع المثبط

خيرات عبد المجيد عليان ، للى صبحى سلامة ،
ألفونس مينا إبراهيم ، تراضى عبد الفتاح ،
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إن التحصين هو الوسيلة لوقاية الحيوانات ضد الأمراض الوبائية ويمكن رفع المستوى
المناعى للحيوانات بإضافة بعض المحسنات للقاحات. وقد استخدم محسن جديد فى هذا البحث
وهو فوسفات الكالسيوم وبمقارنته بالالومنيوم هيدروكسيد جل فى هذا البحث وجد أنه أقوى
ويعطى أجساماً مناعية بنسبة أعلى من الالومنيوم هيدروكسيد جل. بالإضافة إلى أنه مادة
اقتصادية وسهلة التصنيع كما أنها إحدى المكونات الطبيعية فى جسم الحيوان.