EFFECT OF CLOSTRIDIAL ANTIGENS CONCENTRATION ON THE IMMUNE RESPONSE IN SHEEP AND RABBITS

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Abstract

A bivalent vaccine of *Cl.perfringens* types B and D and a polyvalent one containing six antigens (*Cl.perfringens* types B and D, *Cl. septicum, Cl.chauvoei, Cl.novyi* type B and *Cl. tetani* were prepared and concentrated by using ultrafiltration system. The vaccines were tested in rabbits and sheep. The immune response for concentrated vaccines was better than unconcentrated one. Also, formalin consumption was not affected by concentration.

INTRODUCTION

Concentration of a large volume of vaccines is obviously desirable, specially for production of multicomponent vaccine, as the dose should be minimized. The concentration of different antigens could be done either chemically or physically. Ardhali *et al.* (1986) prepared a vaccine against black disease in which the toxoid was concentrated with ammonium sulphate to 20 %, 40%, 60%, and 80% of original volume. Maximum antibody titer was obtained at 80% concentration when the vaccine was tested in rabbits. Published work obtained by many investigators revealed that, clostridial vaccines obtained after purification and concentration of toxins by chemical methods produced antibody titers much higher than the usual vaccines (Tomic 1960, Butazon 1963, Jayaraman and Harbala 1971, Urguer and Ataev 1977, Izumi *et al.* 1983 and Cox *et al.* 1984). Kagan *et al.* (1976) prepared a concentrated polyvalent vaccine from toxoids of *Cl.perfringens* type B, *Cl.septicum* and *Cl.chauvoei* and found that this vaccine gave much higher serum antibody titers than the unconcentrated one.

For large scale production of different vaccines, chemical methods used for concentraation are not practical, so, in this case, ultrafiltration system is preferred as it provides more adequate concentration and purification.

The present work was carried out to evaluate the immune response of concentrated clostridial vaccines (by using ultrafiltration system) and the unconcentrated ones, and to investigate the competition between different antigens in both vaccines.

MATERIALS AND METHODS

Vaccines preparation

Cultures of *Cl.perfringens* types B and D, *Cl.septicum*, *Cl.novyi* type B, and *Cl. chauvoei* were prepared according to Gadalla *et al.* (1974). *Cl.tetani* culture was prepared according to instruction of Rijks (1980). The toxins were separated from the cells and concentrated to the third of their volume by using millipore ultrafiltration system (Millipore Corporation, Bedford Massachusetts, 01730, USA). Toxins power and inactivation time were determined before and after concentration, *Cl.chauvoei* cells were separated and then resuspended in their concentrated filtrate. The average cell counts were enumerated according to Cruickshank *et al.* (1975).

Four vaccine batches were prepared (Table 1), all fulfilled the requirement of the British Veterinary Codex (1970) for safety and sterility.

Table 1. Types and doses of vaccines used.

Vaccine No.	Vaccine type	Injected doses in ml	
		1st dose	2nd dose
I	Concentrated bivalent vaccine of *Cl.perfringens* types B and D	2	1
II	Unconcentrated bivalent vaccine of *Cl.perfringens* types B and D	3	2
III	Concentrated polyvalent vaccine contained *Cl.perfringens* types B and D, *Cl.novyi* type B, *Cl.septicum*, *Cl. chauvoei* and *Cl.testani.**	3	2
IV	Unconcentrated polyvalent vaccine	5	3

* *Cl.tetani* toxoid was added for polyvalent vaccines in 20 Lf/dose

Determination of formalin percentage

Formalin consumption of beta and epsilon toxins of *Cl.perfringens* and alpha toxin of *Cl.novyi* type B were estimated after filtration and concentration according to European Pharmacopoeia (1969).

Immunizing power of prepared vaccines

Four groups each of 10 rabbits and five sheep were vaccinated by the above vaccines. Each animal was injected subcutaneously with 2 doses of vaccine as shown in Table 1 at 4 weeks interval. Rabbits received the same doses of sheep. Blood samples were collected before vaccination and ten days after the second dose. Sera of rabbits were pooled as one sample, but sheep sera were tested individually. Antitoxin titers were determined by serum neutralization test in mice, except for *Cl.chauvoei* antibodies, plate agglutination test was used according to Claus and Macheak (1972). Another two groups of guinea pigs each of 12 were injected with concentrated and unconcentrated polyvalent vaccine, and then, challenged with 32 minimum lethal dose (MLD) of *Cl. chauvoei* spore suspension .

RESULTS

Tables from 2 to 5 illustrated the results obtained.

Table 2. Formalin percentage before and after concentration of toxins.

Type of toxin	Formalin percentage before concentration	Formalin percentage after concentration
Beta toxin of *Cl.perfringens* type B.	0.5%	0.55%
Epsilon toxin of *Cl.perfringens* type D.	0.5%	0.56%
Alpha toxin of *Cl.novyi* type B.	0.5%	0.52%

DISCUSSION

During the preparation of multicomponent vaccine, the antigenic value of each constituent must be increased sufficiently to permit its dilution with other components.

The concentration of antigens in the present work was carried out by using ultrafiltration system, thus, drastic procedures involved in the chemical concentra-

tion was avoided. Also, the formalin percentage was not affected by concentration, as it has been known that high concentration of formalin causes increased destruction of antigen (Who 1978).

Table 3. Toxin values and toxiding time before and after concentration.

Toxins of	Toxins value (MLD/ml)		Inactivation time in days	
	Before concentration	After concentration	Before concentration	After concentration
Cl.perf. type B	1500	7500	12	16
Cl.perf. type D	8000	20.000	11	15
Cl.novyi type B	800	4000	12	17
Cl.septicum	200	1000	10	15

Cl.perf : Cl.perfringens.

Results obtained in Tables 4 and 5 showed that, both concentrated and unconcentrated vaccines gave good response in most types of antigens, but it was somewhat lower in the unconcentrated than in the concentrated one. The response against the epsilon toxoid was much higher in unconcentrated vaccines specially in the bivalent vaccine. This may be due to the nature of the strain used in this work as it was a mutant strain of *Cl.perfringens* type D. It was found also, that, the beta antitoxins titer were the same for the concentrated and unconcentrated in the bivalent, while, it was much higher in concentrated polyvalent vaccine. This may be attributed to the dilution that occurs when components of polyvalent vaccine are mixed. These results disagree with Sterne *et al.* (1962) who found that, diluted unpurified antigens gave better results than chemically concentrated antigens. Also, Kery and Craig (1979) agree with this opinion, and they attributed their finding to the amount of antigen which an adjuvant can adsorb is limited, thus, making the concentration of antigens unnecessary. On the other hand, some authors reported about the advantage of toxoid prepared from purified concentrated toxins. Smith (1957), Ardhali *et al.* (1986), Gupta and Relyveld (1991) and Aly (1996), found that, polyvalent clostridial vaccine obtained after purification and concentration of the toxins produced antibody titres much higher than the usual vaccine.

The possibility of the low response of *Cl.septicum* toxoid may be due to the low toxicity of the strain than to possible interference.

In conclusion, the concentration of antigens has the advantage of reduction of

the doses volume given to the animal, as well as, it produced antibody titer much higher than the usual untreated toxoids. Ultrafiltration system could be recommended as a method which gives more adequate clarification and concentration of antigens used on large scale production of vaccines specially for multicomponent clostridial vaccine.

Table 4. Immunizing power of bivalent vaccine against pulpy kidney and lamb dysentery.

Vaccinated animal	Type of Vaccine	Antitoxic titer (I.U./ml)			
		Beta		Epsilon	
		Prevacc. titer	Postvacc. titer	Prevacc. titer	Postvacc. titer
Rabbit	Conc.	0	19	0	3
	Unconc.	0	14	0	4
Sheep 1	Conc.	0	20	0	7
2		0	18	0	6
3		0	22	0	8
4		0	19	0	8
5		0	16	0	9
Mean		0	19	0	8
Sheep 6	Unconc.	0	21	0	16
7		0	19	0	14
8		0	19	0	14
9		0	18	0	16
10		0	18	0	15
Mean		0	19	0	15

Prevacc. : Prevaccination. Postvacc. : Post-vaccination.
Conc. : Concentrated. Unconc. Unconcentrated.

Table 5. Immunizing power of polyvalent vaccine.

Vaccinated animal	Type of vaccine	Pre-vacc. titer	Antibody titer (I.U./ml)				Immunizing power of *Cl.chauvoei* vaccine	
			Cl. perf. Beta Toxin	Cl. perf. Eps Toxin	Cl. sep. Alpha Toxin	Cl. nov. Alpha Toxin	PAT in ul*	Chal. in G.P S/T
Rabbit	Conc.	0	10	2	3	6	0.05	12/12
	Unconc.	0	5	2.5	2.5	3	0.40	9/12
Sheep 1	Conc.	0	30	3	3.5	19	0.04	
2		0	31	4	2.5	20	0.04	
3		0	32	4	3	21	0.03	
4		0	24	4	3	22	0.05	
5		0	28	5	3	18	0.04	
Mean			30	4	3	20	0.04	
Sheep 1	Unconc.	0	15	5	2.5	10	0.1	
2		0	20	4	2	10	0.1	
3		0	24	5	2.5	12	0.1	
4		0	21	6	1.5	11	0.1	
5		0	20	5	1.5	11	0.1	
Mean			20	5	2	11	0.1	

Eps. : Epsilon. Cl.Sep. : Cl. septicum. Cl.nov. : Cl. novyi.
PAT : Plant Agglutination Test. Chal. : Challenge.
G.P. : Guinea Pig. S/T : Survival / Total.
* The plate agglutination titre was defined as the number of microliter (ul) of serum required to provide definite agglutination (75%) of 0.03 of standard antigen.

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تأثير تركيز انتيجينات الكلوستريديا على الاستجابه المناعية فى الاغنام والأرانب

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تمت دراسة الاستجابه المناعية لنوعين من لقاحات الكلوستريديا هما لقاح دوسنتاريا الحملان والكلوة الرخوة واللقاح الجامع الذى يحتوى على ستة انواع من الانتيجينات وذلك بعد تركيز تلك الانتيجينات بجهاز الترشيح الدقيق.

وقد اوضحت النتائج أن اللقاحات المركزه اعطت نتائج افضل من مثيلاتها الغير مركزه. أيضا لم تؤثر عملية التركيز بتلك الطريقه على نسبه الفورمالين المستخدم فى تثبيط الانتيجينات المستخدمه.