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Immunogenicity of a Gamma-irradiat d Brucella Vaccine

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—국문초록—

Gamma선 조사로 만든 Brucella Vaccine의 생쥐에 대한 면역력

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안 태 휴

Brucella melitensis 균의 치사량($10^6\gamma$)의 Gamma선을 조사해 줌으로써 만든 Vaccine과 가온 또는 화학처치법(ether, formalin, phenol)에 의하여 만든 Vaccine을 생쥐에 접종하여 그 면역성부과능력에 대하여 비교실험 해본 결과, Gamma선 조사에 의하여 만든 Vaccine이 보다 좋은 성적을 가져왔음을 알게 되었다.

생균 Vaccine인 Brucella abortus strain 19과 Brucella melitensis의 R-form을 대량주사 해주었을때 생쥐에 치명적이었으며, 7종의 adjuvant에 대한 효력비교실험은 Freund's complete adjuvant와 aluminum-potassium sulfate와 pectin을 섞여 만든 adjuvant를 제외하고는 그리 의의있는 차이를 발견하지 못했다.

Although Brucella abortus strain 19 was developed as an effective vaccine against brucellosis in cattle, no effective vaccine is available for human protection against brucella infections. Caprine brucellosis is also a serious economic and health problem in many parts of the world. Heat and chemically inactivated Brucella vaccines have not proved to be effective immunogenic agents.

In recent studies we have shown that *B. melitensis* and *B. abortus* can be irradiated with cobalt-60, rendering them non-reproducing and non-infectious. It does not make them incapable of respiring on suitable substrates. [1,3] The present study was carried out to determine the immunogenicity of such gamma-irradiated *B. melitensis* vaccine (GIV) in mice.

MATERIALS AND METHODS

Test organisms: A virulent strain of *B. melitensis* was used both for preparing the vaccine and for challenging the mice. The stock culture was maintained by twice weekly subculturing on albimi agar slants. The mice used were purchased locally.

Preparation of the vaccine: Albimi agar slants were inoculated from stock cultures by means of a platinum inoculating loop and incubated for 48 hours. The cells were washed off the slants with 5 percent Dubos albumin (Difco) solution and pooled in large screw-capped test tubes. The cell suspension was centrifuged, the supernatant fluid discarded, and the cells were resuspended in minimum volume of fresh 5

percent Dubos albumin solution, distributed in approximately 2ml amounts into tared ampoules, and lyophilized in a freeze dryer. Cell suspensions which were not immediately lyophilized were kept frozen in the ampoules until lyophilized. The ampoules with the lyophilized cells were irradiated by exposure to a cobalt-60 source. The heat-treated vaccine was prepared by exposure of the cell suspension to 60 C for 10 minutes in a water bath, and the three chemically inactivated vaccines, by 72-hour exposure at 5 C to 20 percent ether, 1 percent formalin, or 2 percent phenol.

Immunogenicity studies: Vaccines exposed to 10^6r were used in three studies. The lyophilized irradiated cells were resuspended in distilled water in various concentrations. Groups of mice, approximately 2 months of age, were vaccinated subcutaneously with 0.3 ml volume of the vaccine suspensions and challenged 2 weeks later by intraperitoneal injections of 0.2 ml of a suspension containing approximately 5×10^8 or 5×10^9 cells.

Comparative studies were also carried out with vaccines prepared by treatment of cells with heat, ether, formalin, or phenol, and a study was conducted on the ability of various adjuvant mixtures to enhance the protective activity of the gamma-irradiated vaccine (GIV) in mice. The following adjuvant mixtures, mixed in equal proportions with the vaccine suspension, were tested: Freund's incomplete, Freund's complete, 2 percent aluminum hydroxide, 2 percent aluminum hydroxide plus 1 percent n-hexadecane, 5 percent aluminum-potassium sulfate, 2 percent pectin, and 2.5 percent aluminum-potassium sulfate plus 1 percent pectin.

RESULTS

The results are presented in the following Tables 1 to 4. Single vaccine injections of from 0.8 to 0.0001 mg, with and without adjuvant, were highly protective for mice challenged with doses of the homologous *B. melitensis* which killed more than half of the unvaccinate controls within 24 hours (Table 1). The survival rates in the tables are recorded at 1 week after challenge. Comparative studies with the heat and chemically inactivated *B. melitensis* vac-

Table 1. Protective effect of Brucella GIV in mice

Vaccine dosage	Vaccine only			Vaccine plus adjuvant		
	Total No. of mice	No. dead	% survival	Total No. of mice	No. dead	% survival
0.8 mg	7	0	100**	—	—	—
0.4	24	2	92**	27	0	100**
0.2	14	0	100**	14	0	100**
0.1	14	1	93**	14	0	100**
0.05	14	1	93**	21	0	100**
0.025	14	1	93**	21	1	95**
0.01	14	3	79**	14	3	79**
0.001	14	4	72**	14	4	72**
0.0001	14	5	64**	14	9	38**
0(Control)	40	36	10	28	27	4

**P = < .001

***P = > .01, < .02

Table 2 a. Comparative study of various Brucella vaccines in mice

Vaccines (B. melitensis)	Dosage (mg)	Total No. of mice	No. dead*	% Survival*
Gamma-irradiated	1.6	28	7	75
Ether-killed	1.6	28	23	18
Formalin-killed	1.6	28	17	39
Phenol-killed	1.6	28	16	43
Heat-killed	1.6	28	24	14
Untreated (Control)	0	28	28	0

* At one week after challenge; challenge dose $=5\times10^9$

Table 2 b. Statistical compatison of data in Table 2 a-p-values

Vaccines	Untreated	Heatkilled	Phenolkilled	Formalin killed	Etherkilled
Gamma-irradiated	$<.001$	$<.001$	$>.01,\ <.02$	$<.01$	$<.001$
Ether-killed	$>.01,\ <.02$	$>.05$	$>.02,\ <.05$	$>.05$	
Formalin-killed	$<.001$	$>.05$	$>.05$		
Phenol-killed	$<.001$	$>.01,\ <.02$			
Heat-killed	$>.02,\ <.05$				

Table 3. Comparative study of Brucella vaccines in mice

Vaccine	Vaccine dosage	Total No. of mice	No. dead		% Survival*
			pre-challenge	post-challenge	
B. melitensis (R-form living)	1×10^{10}	8	3	0	62
	2×10^{9}	8	0	8	0
	7×10^{8}	8	0	8	0
B. abortus (strain 19, living)	1×10^{10}	8	8	0	0
	2×10^{9}	8	1	1	75
	7×10^{8}	8	0	1	87
B. melitensis (heat-killed)	1×10^{10}	8	0	1	87
	2×10^{9}	8	0	2	75
	7×10^{8}	8	0	4	50
B. melitensis (Gamma-irradiated plus Freund's adjuvant)	1×10^{10}	8	0	0	100
	2×10^{9}	8	0	0	100
	7×10^{8}	8	0	0	100
Untreated (Control)	0	8	0	8	0

* At one week after challenge; challenge dose$=5\times10^9$.

cines demonstrated them to be less effective (Tables 2a and 2b). Living B. abortus strain 19 and living R-form of *B. melitensis* showed some immunogenic activity, but at higher doses were themselves lethal for mice (Table 3).

Statistical comparisons of the adjuvant activity of the seven mixtures tested with the GIV showed no significant differences among them (Table 4). Freund's complete adjuvant and a mixture of aluminum-potassium sulfate with

Table 4. Comparative study of various adjuvants with Brucella gammairradiated vaccine in mice.

Adjuvant*		Total No. of mice	No. ** dead	% Survival
Freund-incomplete		28	2	93
Freund-complete		28	0	100
2% $Al(OH)_3$		28	3	89
2% $Al(OH)_3$	1% n-$C_{10}H_{22}$	28	2	93
5% $Al(SO_4)_3$ K_2SO_4		28	3	89
2.5% Al $(SO_4)_3$ K_2SO_4	1% pectin	28	0	100
1% pectin		28	4	86
No adjuvant-vaccine only		28	7	75
No treatment (Control)		28	28	0

* Adjuvant was mixed with equal volume of vaccine suspension therefore percentage of chemical in final mixture is one-half of stated value. Vaccine dosage=1.6 mg per mouse.

** At one week after challenge; challenge dose=5×10^8.

pectin appeared to enhance the activity of the GIV, but the other adjuvants had no statistically significant effect. The long-term study showed that the immunity developed by 0.4 mg GIV with aluminum hydroxide adjuvant is effective as early as the 4 th day after vaccination and persists as long as 4 months.

DISCUSSION

Although no brucellosis case has been reported in Korea, a considerable number of the patients has been reported in other countries[19].

Calfhood vaccination with the attenuated *B. abortus* strain 19 has done much to reduce the incidence of Bangs disease in cattle. Unfortunetely strain 19 remains pathogenic for man, and the organism has been reported to be recoverable from the products of vaccinated cows. [4,20]

It also affords little or no protection against infection with *B. suis* and *B. melitensis*. Several other promising vaccines against brucellosis are reported to be under investigation, [5-8,12,14-18] but none has proved in extensive field trials to be safe and effective.

The ideal vaccine against any infectious disease is an agent antigenicably identical with the causative organism but without the ability to produce disease. Radiation treatment of virulent cells was selected as a possible means of approaching this ideal on the basis of studies which indicated that many cells respond to proper doses of radiation by undergoing mitotic or reproductive death, much larger doses being required to produce "interphase" death [2]. Our own studies have shown that 10^6 r cobalt-60 irradiation of lyophilized Brucella renders the cells incap able of producing visible colonies when cultured on albimi agar, yet these cells continue to respire on suitable substrates at approximately 50 percent of the rate of nonirradiated cells capable of normal reproduction. Such irradiated cells are likely to be less damaged than cells subjected to heat or chemical treatment, and genetically and antigenically are more similar to the virulent cells than attenuated nonvirulent strains.

The question of substrate reactivation of radiation-inactivated cells has been raised in literature and widely debated [9-11,13]. Studies have indicated that irradiated cells which have undergone mitotic death may divide several times in favorable medium [2], but any further growth is an indication that the cells have not undergone mitotic death. The possibility that irradiated

cells, which do not visibly multiply on cultiply on culture medium, may be reactivated in the animal host was explored, but all of our tests with the Brucella GIV have shown that such cells are incapable of producing disease in experimental animals, or of being recovered from spleen cultures of GIV-vaccinated mice. The Brucella GIV cells may be quite capable of dividing once or twice or even four or five times, and such limited multiplication in the animal may play an important role in the production of immunity, but these cells certainly do not continue to propagate to the point of producing evidence of infection.

The postchallenge survival of some of the mice given infections of spleen homogenate from vaccinated mice may be interpreted as passive transfer of immunity or even transfer of some of the GIV cells concentrated in the spleen of the vaccinated mice, inasmuch as these mice received 0.1 mg of GIV and as little as 0.0001 mg was shown to give some protection, but the negative spleen cultures and the failure to transfer immunity with spleen homogenates of the mice receiving the homogenate from the vaccinated mice obviate the interpretation that this transfer of immunity is due to any extensive proliferation of reactivated GIV cells. The number of animals in each group of this experiment is too small for the results to be conclusive. Further studies along these lines may be of interest in elucidating the mechanism of the immunogenic activity of GIV.

SUMMARY

A vaccine was prepared by 10^6 r cobalt-60 irradiation of lyophilized virulent *Brucella melitensis* and tested in mice for immunogenic activity against a lethal challenge dose of the homologous strain. The vaccine (GIV) produced a high degree of immunity in mice, and comparative studies demonstrated it to be superior to vaccines prepared by heat or by chemical (ether, formalin, or phenol) treatment. Living vaccines, *Brucella abortus* strain 19 and an R-form of *Brucella melitensis* were lethal for mice in larger doses. A comparison of seven adjuvant mixtures for use with the GIV showed no statistically significant difference, but Freund's complete adjuvant and a mixture of aluminum-potassium sulfate and pectin appeared to enhance the activity of the GIV.

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