EFFECT OF LEVAMISOLE AS IMMUNOPOTENTIATOR ON THE IMMUNE RESPONSE OF *BRUCELLA ABORTUS* STRAIN 19 VACCINATED GUINEA PIGS

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Abstract

Studies were directed to evaluate the effect of levamisole on the vaccinated guinea pigs with *Brucella abortus* strain 19 vaccine when injected simultaneously or post- vaccination. It was found that levamisole was improving the level of antibodies titre in sera of treated group compared with non-treated one. In challenge test for vaccinated group, a good protection was observed in animals that received levamisole either simultaneously with the vaccine or post-vaccination.

INTRODUCTION

Brucellosis is a highly contagious disease affecting animals causing economic losses. Vaccination of animals with *Brucella abortus* strain 19 vaccine is found to provide protection against the disease (Radostitis *et al.,* 2000).

Many drugs and chemicals can enhance various aspects of the immune response, non-specific immunostimulants are going increased attention and attraction for application in the veterinary medicine to help the animal in its struggle against disease through potentiation of its immune response to the applied vaccine (Clark, 1991). The use of immunostimulant agent together with *Brucella* vaccine might be more effective than a single measure, and raise animal resistance to infection (Arnault, 1981 and Chukwu, 1985). Levamisole is a safe broad-spectrum anthelmintic. Since the discovery of this drug, numerous reports have confirmed the suggestion that levamisole affects both humoral and cellular immune response and increases the resistance to bacterial infection particularly in previous immunized host (Wierda and Reasor, 1990).

The present investigation was planned to gain more explicit information on the effect of using levamisole on the immune response of guinea pigs vaccinated with *Brucella abortus* strain 19 vaccine.

MATERIALS AND METHODS

Brucella vaccine

A locally produced *Brucella abortus* strain 19 vaccine obtained from Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo was used in vaccination of guinea pigs.

Brucella antigens

A locally produced Brucella agglutinating and Rose Bengal antigens obtained from Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo were used in serological tests.

Challenging _Brucella_ strain

The standard Brucella strain 2308 obtained from Ames, Iowa, USA was used in challenge test.

Levamisole

It was obtained from Amoun Pharmaceutical Industries Company, Cairo.

Guinea pigs

A total of one hundred guinea pigs (350g each), proved to be *Brucella* free by serotesting was used for the experiment.

Experimental designs

Guinea pigs were divided into 5 groups (20 each), group (1) given levamisole intramuscularly (one dose of 0.1 ml) 3 days before vaccination with *Brucella abortus* vaccine (1/15 dose of 3-10 x 10^9 cfu/dose); group (2) given levamisole at the same time of vaccination; group (3) given levamisole 7 days after vaccination; group (4) was vaccinated without any treatment, while, group 5 was left as control group (unvaccinated and non-treated). Blood samples were taken from each animal before and after vaccination and treatment at weekly intervals till the 8^{th} week. The immune response of animals was evaluated by applying the Rose Bengal plate test according to Morgans *et al.* (1969), and tube agglutination test according to Alton *et al.* (1988). At the 8^{th} week post-vaccination, all animals were challenged subcutaneously with a 24 hour broth culture of *Brucella* strain 2308 (5 x 10^6 cfu/dose); also, untreated vaccinated group and control group were similarly challenged. Blood samples were taken after 1 week till 8^{th} week of challenge. All animals were sacrificed after 8 weeks of challenge and the CFU in their spleens was determined, and calculation of protection percent was carried out according to British Pharmacopoeia Veterinary (2005).

RESULTS AND DISCUSSION

The most reliable method for controlling Brucellosis is usually afforded by vaccination with *Brucella abortus* strain 19 vaccine (Radostitis *et al.*, 2000). For successful prophylactic immunization against Brucellosis, the use of vaccine should produce adequate immunization. The use of immunostimulant besides vaccination may lead to increased protection against Brucellosis (Sandoval *et al.*, 1978).

The immune response of vaccinated animals with vaccine treated with or without levamisole was screened by Rose Bengal and tube agglutinating tests. These

methods assayed anti-Brucella antibodies in sera of animals at different intervals post-vaccination and treatment as shown in Table 1. These antibodies began to appear in the sera of animals from 2nd week post-vaccination, and higher titre was recorded by 5th week except in groups 2, 3 by 8th week. From this table, levamisole is enhancing the production of antibodies titre. Possible reasons for the previous stimulant activity of levamisole are direct increase in the antibodies forming cells in the spleen and indirect stimulation of B-cells through T-cells mediators (Renoux and Renoux, 1972 and Koller, 1982). Also, such finding was obtained by Renoux and Renoux (1973) who found that levamisole injected in mice (at time of vaccination or 48 hours later) induced a significant increase of antibodies titre of *Brucella* vaccine. On keeping with these lines, Confer *et al.* (1985) recorded that levamisole given 7 days after vaccination caused enhancement of antibodies response to *Brucella* vaccine. Furthermore, Chukwu (1985) indicated that heifers vaccinated simultaneously with levamisole showed increase in humoral antibodies titre. With respect to previous findings, the following explanation proves that levamisole stimulates the multiplication of *Brucella* vaccine in treated group as well as its immune stimulated effect (Milward *et al.*, 1984). The results summarized in Table 2 indicated the protective efficacy of levamisole in challenged animals. The results showed a low titre level in sera of treated animals after challenge. These results are agreed and support that recorded previously by Koller (1982) who revealed levamisole as immunostimulant. This drug enhanced the cellular immune response to the impact of vaccine, hence, those animals will offer more resistance to infection with *Brucella* infection. It has been demonstrated that a lower *Brucella* abortus isolation was associated with guinea pigs vaccinated and treated with levamisole. The previous increase of protection level against infection with brucellosis in treated group is attributed to an augmentation of lymphocyte and macrophage proliferation, phagocytosis and increase of lysosomal enzymes related with increase of the intracellular killing. Furthermore, levamisole stimulates cytotoxic T-cells associated with enhancement of lymphokine production. The previous concept draw attention to an increase in the rate of clearance of infective agents (Brunner and Muscoplat, 1980). The results summarized in Table 3 indicated the protective efficacy of the levamisole when injected simultaneously or post-vaccination of *Brucella* vaccine strain 19 in challenged test. The table shows that guinea pigs gave protection level of 85% in vaccinated group, 90% in treated group simultaneously with vaccine and 90% in treated group 7 days post-vaccination. The obtained results are agreed and support that recorded previously by Sandoval *et al.* (1978) who found that the post-vaccination injection of levamisole in guinea pigs revealed a higher level of protection with vaccination.

In conclusion, levamisole can be used at vaccination or post-vaccination 7days for enhancing the immune system.

Table 1. Immune response of vaccinated guinea pigs with Brucella abortus S19 vaccine measured by Rose Bengal plate test (RBPT) and serum agglutinating tests (SAT).

Groups	Tests	Weeks Post Vaccination							
		1	2	3	4	5	6	7	8
Group (1) received levamisole 3 days before vaccination	RBPT	-	+	+	++	++	++	++	+
	SAT	0	20 IU	40 IU	80 IU	160 IU	80 IU	80 IU	40 IU
Group (2) received levamisole at the time of vaccination	RBPT	-	+	++	++	+++	+++	++	++
	SAT	0	20 IU	80 IU	80 IU	160 IU	160 IU	80 IU	80 IU
Group (3) received levamisole 7 days after vaccination	RBPT	0	+	++	+++	++++	+++	++	++
	SAT	-	40 IU	80 IU	160 IU	320 IU	160 IU	80 IU	80 IU
Group (4) received vaccine only	RBPT	-	+	+	+	++	++	+	+
	SAT	0	20 IU	40 IU	40 IU	80 IU	80 IU	40 IU	20 IU
Group (5) without vaccine or levamisole (Control group)	RBPT	-	-	-	-	-	-	-	-
	SAT	0	0	0	0	0	0	0	0

IU: International Unit

Table 2. Immune response of challenged vaccinated guinea pigs measured by Rose Bengal plate test (RBPT) and serum agglutinating tests (SAT).

Groups	Tests	Weeks Post Vaccination							
		1	2	3	4	5	6	7	8
Group (1) received levamisole 3 days before vaccination	RBPT	-	+	+	++	++	+	+	+
	SAT	0	10 IU	20 IU	40 IU	40 IU	20 IU	10 IU	10 IU
Group (2) received levamisole at the time of vaccination	RBPT	-	+	+	+	+	+	-	-
	SAT	0	10 IU	20 IU	20 IU	20 IU	10 IU	-	-
Group (3) received levamisole 7 days after vaccination	RBPT	-	-	+	+	+	+	-	-
	SAT	0	0	10 IU	20 IU	20 IU	10 IU	-	-
Group (4) received vaccine only	RBPT	-	+	+	++	++	+	+	+
	SAT	0	10 IU	20 IU	40 IU	40 IU	20 IU	20 IU	10 IU
Group (5) without vaccine or levamisole (Control group)	RBPT	-	+	++	++	++	++	++	+
	SAT	0	20 IU	40 IU	80 IU	160 IU	80 IU	40 IU	20 IU

IU: International Unit

Table 3. Protection percent of guinea pigs vaccinated with *Brucella vaccine* and treated with levamisole after challenge with virulent *Brucella abortus* strain 2308.

Group	Number of animals	Protection percentage	
		S/N	%
Group (1) received levamisole 3 days before vaccination	20	3/20	85 %
Group (2) received levamisole at the time of vaccination	20	2/20	90 %
Group (3) received levamisole 7 days after vaccination	20	2/20	90 %
Group (4) received vaccine only	20	3/20	85 %
Group (5) without vaccine or levamisole (Control group)	20	20/20	0 %

S: Spleen containing Brucella strain.
N: Number of challenged animal.

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REFERENCES

1. Alton, C. C., J. M. Jones, R. D. Ongus and S. M. Verger. 1988. Technique for Brucellosis. Laboratory Institute National de la Recherche Gronomique (1 NRA), 1777 Rue de li, Universte 75007, Paris.
2. Arnault, G. 1981. Production of immediate antibodies after vaccination of heifers with S-19: Effect of some intercurrent factors (disease, use of immunostimulants such as levamisole and genitic). Bulletin Mensule de la Societe Veterinaire Practique defrance, 65 (6): 434-461.
3. British Pharmacopoeia, Veterinary. 2005. Veterinary Vaccines, 162-163, London Her Majesty, Stationary Office.
4. Brunner, C. J. and C. C. Muscoplat. 1980. Immunomodulatory effect of levamisole. J. Am. Vet. Med. Ass., 176, 10 (2): 1159-1162.
5. Chukwu, C. C. 1985. Serological response of cattle following Brucella abortus strain 19 vaccination and simultaneous administration of levamisole. International J. Zoonosis, 3 (12): 196-202.
6. Clark, W. R. 1991. The experimental foundation of modern immunology. 4th ed. Chisteri John Wiley and Sons, USA.
7. Confer, A. W., S. M. Hell and B. H. Espa. 1985. Transient enhancement of serum antibody response to Brucella abortus strain 19. Am. J. Vet. Res., 46: 2440-2443.
8. Koller, L. D. 1982. Chemical induce immunomodulation. JAVMA, 10: 1102-1106.
9. Milward, F. W., P. Nicoletti and L. Hoffmann. 1984. Effectiveness of various therapeutic regimens for bovine Brucellosis. Am. J. Vet. Res., 45: 1825-1828.

10. Morgans, E. C., D. J. Mackion and C. A. Cullan. 1969. The Rose Bengal Plate agglutination in diagnosis of Brucellosis. Am. J. Vet. Res., 85: 636-641.
11. Radostitis, Q. M., C. C. Gay, D. C. Blood and K. W. Hinchcliff. 2000. Veterinary Medicine. Textbook of the Disease of Cattle, Sheep, Pigs, Goats and Horses. 9th ed. W.B. Saunders Company LTD.
12. Renoux, G. and M. Renoux. 1972. Antigenic competition and non-specific immunity after a ricketsial infection in mice: restoration of antibacterial immunity by phenyl-imidothiazole treatment. J. Immunol., 109 (4): 761-765.
13. Renoux, G. and M. Renoux. 1973. Stimulation of antibrucella vaccination in mice by tetramisole, phenyl imidothiazole salt. Infect. Immun., 8: 544-549.
14. Sandoval, L. A., W. Giorgi, I. B. S. Amaral, E. Zomplli and M. T. Manzati. 1978. Immunostimulating effect of levamisole in the immunization of guinea pigs against brucellosis. Aquivos do Instituto Biologica, sampaulo, Brazil, 45 (4): 313-317.
15. Wierda, D. and M. J. Reasor. 1990. Immunomodulating drugs. Cited in Modern Pharmacology. 3rd ed. edited by Charles, R. Craig and Robert, E. Stizel. 1990. Little Browhand Company Boston, Toronto, London.

تأثير الليفاميزول على رد الفعل المناعى فى الأرانب الهندية المحصنة ضد البروسيلا أبورتس عترة ١٩

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أجريت هذه الدراسة لمعرفة تأثير الليفاميزول على الأرانب الهندية المحصنة بلقاح البروسيلا أبورتس عترة ١٩ عندما يحقن أثناء أو بعد التحصين. وقد أثبتت النتائج أن هناك زيادة معنوية فى مستوى الأجسام المناعية إذا ما قورنت بالمجموعة الغير معالجة. وقد تأكدت تلك النتائج بعمل اختبار تحدى المناعة وكانت نسبة الوقاية ضد العدوى بالميكروب الضارى عالية فى المجموعة المعالجة بالليفاميزول أثناء أو بعد التحصين. بناء على النتائج السابقة يمكن أن يوصى بإمكانية استخدام الليفاميزول أثناء أو بعد التحصين لما له من تأثير منشط للجهاز المناعى.