

RESEARCH NOTE

EFFECT OF LEVAMISOLE ON IMMUNITY TO *CORYNEBACTERIUM PSEUDOTUBERCULOSIS* IN MICE AND SHEEP

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ABSTRACT

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The stimulating effect of levamisole on immunity to *Corynebacterium pseudotuberculosis* in mice was marginal, while no enhancement of immunity could be detected in sheep.

The conclusion was reached that, as levamisole has no potentiating effect on immunity to *C. pseudotuberculosis* in normal sheep, it is of no practical value as an immunostimulant in this instance.

Résumé

L'INFLUENCE DE LA LEVAMISOLE SUR L'IMMUNITÉ DE LA SOURIS ET DU MOUTON À *CORYNEBACTERIUM PSEUDOTUBERCULOSIS*

L'effet stimulant de la levamisole sur l'immunité à *Corynebacterium pseudotuberculosis* chez la souris est faible, tandis qu'aucune accélération de l'immunité ne peut être démontrée chez le mouton.

Puisque ce vermifuge n'a aucune influence favorable sur l'immunité à *C. pseudotuberculosis* chez le mouton normal, sa valeur immunostimulatrice est pratiquement nulle dans ce domaine.

INTRODUCTION

Levamisole hydrochloride, a commonly used anthelmintic, has been shown to enhance the immune response in various experimental systems. Tripodi, Parks & Brugmans (1973) reported that it restored cutaneous delayed hypersensitivity in anergic patients, and Woods, Fliegelman & Chirigos (1975) demonstrated that it stimulated the *in vitro* immune response of spleen lymphocytes, while Fisher, Podgore, Bass, Kelley & Kobayashi (1975) found that suckling rats treated with levamisole exhibited enhanced resistance to challenge with *Staphylococcus aureus* and herpes simplex virus. More specifically, Renoux & Renoux (1973) showed that administration of tetramizole significantly enhanced the immunity of mice immunized with inactivated *Brucella melitensis*. Similarly, Irwin & Knight (1975) reported that levamisole, given with a sublethal infection of *Corynebacterium pseudotuberculosis*, increased the resistance of mice to subsequent challenge with *C. pseudotuberculosis*, despite depressed serum globulin levels.

Although both mice (Cameron & Minnaar, 1969) and sheep (Cameron, Minnaar, Engelbrecht & Purdom, 1972) can be adequately immunized against a lethal infection of *C. pseudotuberculosis*, not all animals are successfully protected against the subsequent development of abscesses (Cameron & Fuls, 1973), and therefore it was proposed to establish whether levamisole would have a potentiating effect on the immunity induced by inactivated *C. pseudotuberculosis* vaccine.

PREPARATION OF VACCINE

Vaccine was prepared essentially as described by Cameron & Minnaar (1969), with some modifications. Mass cultivation of *C. pseudotuberculosis* strain 137B was done in an aerated 400 litre fermentation tank, using a medium composed of a locally produced nutrient broth enriched with lactalbumin (Difco) 10 g/litre and yeast extract (Difco) 5 g/litre (Cameron & Swart, 1965; Cameron *et al.*, 1972). After 48 h incubation at 37 °C, the packed cell volume was determined and the whole culture was harvested, inactivated by the addition of 0.7% formalin and kept for 5 days at 37 °C. The cells were then precipitated with 1% alum and the sediment washed twice

with saline (+0.7% formalin). Finally, the sediment was re-suspended in saline with a 0.7% formalin content to contain 2.5% packed cells.

EXPERIMENTS

Mice

A group of thirty-six 4-6-week-old female albino mice was immunized by 2 subcutaneous injections of 0.2 ml vaccine, with an interval of 4 weeks between the injections. Levamisole (Ripercol-1)* was injected intramuscularly at a rate of 7.5 mg/kg simultaneously with and 48 h after the administration of the vaccine. A 2nd group of 36 mice was similarly immunized except that levamisole was administered at the rate of 1.25 mg/kg. A 3rd group of 36 mice was given vaccine only, while a 4th group served as controls. Challenge material was prepared according to the method described by Cameron & Minnaar (1969), and 10 mice from each group were challenged 10 days after the 2nd injection of vaccine by intravenous injection of 5×10^6 , 1×10^6 and 2×10^5 bacteria, respectively. Deaths were recorded for 14 days. All the non-immunized control animals died within this period.

From the results recorded in Table 1, it would appear that levamisole slightly enhances the immunity which is induced by the vaccine alone.

TABLE 1 Effect of levamisole on immunity to *C. pseudotuberculosis* in mice

Group	Treatment	Challenge dosage/mouse	Protection %	Average protection %
1.....	Vaccine + levamisole 7.5 mg/kg	5×10^6	40	57
		1×10^6	60	
		2×10^5	70	
2.....	Vaccine + levamisole 1.25 mg/kg	5×10^6	60	67
		1×10^6	70	
		2×10^5	70	
3.....	Vaccine only	5×10^6	30	43
		1×10^6	40	
		2×10^5	60	
4.....	None	5×10^6	0	0
		1×10^6	0	
		2×10^5	0	

* Ethnor Laboratories (Pty) Ltd, Johannesburg

A possible explanation for the discrepancy between these results and those of Irwin & Knight (1975) could be the fact that these workers used live organisms for immunization which could have induced a certain degree of cellular immunity. An inactivated vaccine as used in this study generates a serum-antibody-mediated immunity (Cameron & Engelbrecht, 1971) which may not be equally amenable to stimulation by levamisole.

Sheep

Four groups of 9 Merino wethers were used in this part of the investigation. Group 1 was given 2 subcutaneous injections of 5 ml of *C. pseudotuberculosis* vaccine with an interval of 6 weeks between the injections. In addition, levamisole (3 mg/kg) was injected intramuscularly 48 h before, simultaneously with and 48 h after the injection of vaccine. Group 2 was given vaccine only and Group 3 was given levamisole only at the same dosages and levels as Group 1, while Group 4 served as untreated controls.

Bacterial suspensions for challenge purposes were prepared according to the procedure described by Cameron *et al.* (1972), and all the animals were intravenously challenged 2 weeks after the 2nd injection of vaccine with 5.0 ml of the standardized suspension.

Deaths were recorded for 1 month, after which all the surviving sheep were slaughtered and the extent of lung lesions was assessed (Cameron *et al.*, 1972).

The results given in Table 2 clearly show that levamisole had no potentiating effect on the level of immunity induced in sheep by an inactivated *C. pseudotuberculosis* vaccine. On the contrary, sheep which received levamisole appeared to be very slightly more susceptible to infection than those which were not treated.

TABLE 2 Effect of levamisole on immunity to *C. pseudotuberculosis* in sheep

Group	Treatment	Deaths/9 through 30 days	Mean death time: days	Mean abscesses/lung of surviving sheep
1.....	Vaccine + levamisole	0	—	57
2.....	Vaccine only	0	—	16
3.....	Levamisole only	6	4,7	91
4.....	None	7	6,7	85

CONCLUSIONS

In spite of the slight effect observed in mice, which agrees with the results obtained with immunity to *Salmonella dublin* in mice (Cameron & Fuls, 1976), the claims of other authors regarding the adjuvant effect of levamisole could not be substantiated.

The results obtained confirm previous findings that sheep can be successfully immunized against infection with *C. pseudotuberculosis* provided that a vaccine of adequate concentration is used (Cameron & Fuls, 1973). The degree of immunity which can be induced is not improved, however, by the administration of levamisole, and any effect which this drug may have in normal animals is so small as to be of no practical consequence whatever.

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REFERENCES

- CAMERON, C. M. & ENGELBRECHT, MARIA, M., 1971. Mechanisms of immunity to *Corynebacterium pseudotuberculosis* in mice using inactivated vaccine. *Onderstepoort Journal of Veterinary Research*, 38, 73-82.
- CAMERON, C. M. & FULS, W. J. P., 1973. Studies on the enhancement of immunity to *Corynebacterium pseudotuberculosis*. *Onderstepoort Journal of Veterinary Research*, 40, 105-114.
- CAMERON, C. M. & FULS, W. J. P., 1976. Immunization of mice and calves against *Salmonella dublin* with attenuated live and inactivated vaccines. *Onderstepoort Journal of Veterinary Research*, 43, 31-38.
- CAMERON, C. M. & MINNAAR, J. L., 1969. Immunization of mice against *Corynebacterium pseudotuberculosis*. *Onderstepoort Journal of Veterinary Research*, 36, 207, 210.
- CAMERON, C. M., MINNAAR, J. L., ENGELBRECHT, MARIA, M. & PURDOM, MARY, R., 1972. Immune response of Merino sheep to inactivated *Corynebacterium pseudotuberculosis* vaccine. *Onderstepoort Journal of Veterinary Research*, 39, 11-24.
- CAMERON, C. M. & SWART, C. F., 1965. A new liquid medium for the cultivation of *Corynebacterium pseudotuberculosis*. *Journal of the South African Veterinary Association*, 36, 185-188.
- FISCHER, G. W., PODGORE, J. K., BASS, J. W., KELLEY, J. L. & KOBAYASHI, G. Y., 1975. Enhanced host defence mechanisms with levamisole in suckling rats. *Journal of Infectious Diseases*, 132, 578-581.
- IRWIN, M. R. & KNIGHT, H. D., 1975. Enhanced resistance to *Corynebacterium pseudotuberculosis* infections associated with reduced serum immunoglobulin levels in levamisole-treated mice. *Infection and Immunity*, 12, 1098-1103.
- RENOUX, G. & RENOUX, MICHELENE, 1973. Stimulation of anti-*Brucella* vaccination in mice by Tetramisole, a phenyl-imidothiazole salt. *Infection and Immunity*, 8, 544-548.
- TRIPODI, D., PARKS, M. D. & BRUGMANS, J., 1973. Drug-induced restoration of cutaneous delayed hypersensitivity in anergic patients with cancer. *New England Journal of Medicine*, 289, 354-357.
- WOODS, WILNA, A., FLIEGELMAN, M. J. & CHIRIGOS, M. A., 1975. Effect of levamisole on the *in vitro* immune response of spleen lymphocytes. *Proceedings of the Society for experimental Biology and Medicine*, 148, 1048-1050.