A novel approach to vaccination against the sheep blowfly

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Summary

Vaccination against the sheep blowfly represents an alternative approach to controlling this important ectoparasite. In order for vaccination to be effective appropriate target molecules must be identified and evaluated. Insect hormones represent potential targets for vaccination due to their importance in larval development. A vaccine trial was therefore undertaken in which sheep were vaccinated with the hormone 20-hydroxyecdysone (20-HE) and subsequently challenged with blowfly larvae. There was a 27% reduction in the weight and a 23% reduction in the number of larvae recovered from the vaccinated sheep compared to the controls. This difference was not statistically significant at the 5% level due to the variation in the response between sheep. Vaccinated sheep mounted a humoral response to the hormone as assessed by ELISA. In addition, electron microscopy studies of larvae recovered from vaccinated sheep indicated that new cuticle formation appeared to have been disrupted. These effects were not observed in larvae recovered from control sheep. The potential for hormones to be used as targets for vaccination will be discussed.

Keywords

Sheep blowfly, vaccination, antigens, hormones

Introduction

There is a need to develop new treatments against the sheep blowfly and vaccination represents a potential option to solving this problem. Vaccination is a non-chemical form of treatment and would therefore alleviate problems associated with the continued use of these agents. Finally, a vaccine effective against breech strike would also reduce the need for mulesing thereby improving animal welfare. The development of a vaccine against blowfly is however critically dependent on identifying appropriate target molecules in the larvae. Recent research in this area has identified a number of candidate blowfly vaccine molecules, however the unusual biochemical nature of these molecules led to difficulties in purification, expression and subsequent testing (Bowles, 2001) (these proceedings). In order to advance this important area of vaccine research, new potential targets must be identified.

Recent research in our laboratory has investigated potential for developing a blowfly vaccine based around molecules that are involved in moulting of the larvae. This approach has a number of major advantages over previous vaccination attempts. Firstly, the process of moulting is critical for insect development and therefore any disruption of this process is likely to have major effects on larval survival. Secondly, several of the key molecules involved have been identified, characterised and are available in quantity from commercial suppliers enabling large numbers of sheep to be tested. Finally, these molecules are also known to be involved in egg formation, maturation and metamorphosis adding to their attractiveness as vaccine targets. This approach represents a novel strategy to parasite control that has not been previously exploited. The following trial describes the use of an insect hormone as a target in an attempt to vaccinate sheep against flystrike.

Methods

Vaccination with 20-HE

A vaccination trial was undertaken using the moulting hormone 20-HE. Sheep were vaccinated subcutaneously with 100μg of the hormone coupled to the carrier KLH. The hormone/carrier complex was added to Montanide ISA-25 containing recombinant IL-1β as adjuvant. Control sheep received the adjuvant only. All sheep were vaccinated three times and then challenged

with 300 *L. cuprina* larvae for 48 hrs after which time the larvae were removed, weighed and counted.

Serum antibody response to 20-HE

Sera was collected prior to the primary vaccination and one week post the third vaccination for analysis by ELISA. A standard ELISA protocol was used that involved coating plates with 1μg per well of either 20-HE coupled to KLH or 20-HE couple to BSA. This enabled the specific response to both the hormone and to KLH to be determined.

Electron microscopy

Following removal from both vaccinated and control sheep, the larvae were fixed and prepared for analysis by electron microscopy.

Results

The results of the challenge experiment are given in Table 1.

Table 1. Results of the challenge experiment.

Group	Av. larval weight (mg)	Av. larval number	% reduction in larval weight compared to controls	% reduction in larval number compared to controls
Vaccinates (n=5)	0.55 (0.3)*	116 (42)*	27#	23#
Controls (n=10)	0.75 (0.49)*	151 (77)*	-	-

*numbers in brackets refer to standard error of the mean
P>0.05

The results of this vaccination trial indicated that there was a 27% reduction in the average weight of the surviving larvae recovered from vaccinated compared to control sheep and a 23% reduction in the average number of larvae recovered from vaccinated versus control sheep.

Vaccinated sheep produced antibodies to the hormone when 20-HE was coupled to the irrelevant carrier, BSA. Vaccinated sheep also produced antibodies to KLH. In an attempt to determine whether there were any changes to the moulting process induced through vaccination, larvae recovered from both vaccinated and control sheep were analysed by transmission electronmicroscopy. The results of these studies indicated that new cuticle formation appeared to have been disrupted in larvae taken from the vaccinated sheep. These effects were not observed in larvae recovered from control sheep. There appeared to be a detachment of the epidermal cells from the endocuticle indicating disruption in the ability in these larvae to form a new cuticle.

Discussion

The process of moulting is critical for insect development and therefore any disruption of this process is likely to have major effects on larval survival. Moulting in insects is controlled by a number of different hormones and proteases. The three key hormones involved are juvenile hormone (JH), ecdysone and 20-hydroxyecdysone (20-HE). The aim of this approach is to generate antibodies to block the action of 20-HE and thereby interfere with the processes that require this hormone. 20-HE is known to act on the central nervous system of insects inducing cellular responses including neuronal proliferation, maturation, cell death and remodelling of larval neurons. In addition, these molecules are known to be involved in egg formation, maturation and metamorphosis adding to their attractiveness as vaccine targets. This approach represents a novel form of vaccination against this parasite. The experiment described above indicates that anti 20-HE antibodies were generated in sheep following vaccination. In addition, the results indicate that vaccination is able to disrupt normal cuticle formation in the larvae and thereby affect their normal development. One major advantage of this approach is that these

molecules are available from commercial suppliers enabling large scale trials to be conducted. Further studies are required to more fully evaluate this novel approach to vaccination examining the use of different carriers, coupling technologies and choice of hormone or protease.

Conclusion

The use of insect hormones, hormone analogues or proteases offers the potential to develop new approaches to vaccination against the sheep blowfly. This approach involves the generation of circulating antibodies to a defined target that are able to affect circulating levels of the hormone in the larval haemolymph. The ability to disrupt the moulting process represents an exciting possibility for future attempts at controlling the sheep blowfly through vaccination.

References

Bowles V. (2001). Progress on vaccination against the sheep blowfly. (*these proceedings*)