1 ABSTRACT

Malaria is a major public health problem for many countries. The present study examines the potential of antisense therapy for controlling the expression of *Plasmodium falciparum* genes and a preliminary step in developing DNA vaccine for malaria - experimental DNA immunisation of rabbits with a candidate vaccine antigen.

The effects of antisense and sense oligodeoxynucleoside phosphorothioates (ODN-S) based on *P. falciparum dihydrofolate reductase- thymidylate synthase* (*DHFR-TS*) and *45 kDa merozoite surface antigen* (*MSA2*) mRNA on malaria parasite growth were examined *in vitro*. The effects of the inhibitors on parasite growth and invasion were assessed by counting different life stages of the parasite under light microscope (x1000) and by [^{3}H]-hypoxanthine incorporation. Antisense and sense ODN-S based on both genes inhibited only the schizont to ring invasion of merozoites into red blood cells but not maturation of rings to trophozoites and schizonts. The effects of ODN-S were non-sequence specific but dose dependent manner. The reduction in the number of ring stage parasites after 24h indicated the inhibition of invasion of merozoites into red blood cells. At 10 μM, the ODN-S inhibited 66-99% invasion.

Exposure of parasites to 0.1 and 1.0 μM ODN-S in culture for six days involving three cycles of invasion into red blood cells also did not demonstrate specific antisense effects. The control ODN-S and a known polyanion, dextran sulphate also produced similar inhibitory effects on parasite growth *in vitro* and this further supported the sequence - independent effects of ODN-S.

When the negative charges of the ODN-S were neutralised by binding to cationic lipid liposomes, they prevented the inhibition of merozoite invasion. Therefore the ODN-S, because of their polyanionic nature, interfere with the binding of merozoites to receptors

of the red blood cells. Hence clinically acceptable polyanions may be used for malaria therapy.

DNA vaccination can potentially overcome many of the drawbacks of the conventional vaccine methods. The coding sequences for two N-terminal fragments (1326 and 1386 bp) of the *P. falciparum* 185 kDa merozoite surface protein (*MSA1*), which is considered as a potential vaccine candidate against malaria were amplified by polymerase chain reaction and cloned into eukaryotic expression vectors VR1020 and VR1012 to yield plasmids P3 and P4 respectively. The MSA1 signal peptide sequence, present in P4, was replaced with the human tissue plasmogen activator signal sequence in P3. The genes in both plasmids were expressed under the control of the cytomegalovirus promoter and intron A enhancer and carried 3' bovine growth hormone termination/poly A signals. Both constructs were able to express MSA1 polypeptide when transfected into Cos cells. Protein expression in the Cos cells by htPA construct did not differ from that of non htPA plasmid construct in western blots. Intramuscular and intradermal inoculation of rabbits with both plasmids have elicited antibodies to MSA1 when analysed by IFA, titres ranging from 10^{-2} - 10^{-3}. These antibody levels were maintained in rabbits for more than 9 weeks after the final immunisation and at 48 weeks it faded almost to the background. However antibodies of an immunised rabbit with the IFA titre of 10^{-3} did not detectably inhibit the growth of *P. falciparum in vitro*. However, these observations with DNA immunisation experiments indicate the potential for developing a DNA vaccine for malaria.