ANALYSIS OF TWO PUTATIVE CANDIDATE GENES FOR ISOMETAMIDIUM RESISTANCE IN *TRYPANOSOMA CONGOLENSE*

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ABSTRACT

The efficacy of the few available trypanocides is being seriously compromised by the emergence and spread of drug resistant trypanosome strains. Elucidation of the molecular genetic basis of this phenotype may contribute to an understanding of the mechanisms of development of resistance to the trypanocides. This knowledge can be exploited for the rational design of drugs to which the parasites may not easily develop resistance.

In order to investigate genetic mechanisms underlying the isometamidium chloride resistance in African trypanosomes, a clone of *Trypanosoma congolense* was made 200-fold more resistant to the drug by continued subcurative treatment. Comparative differential display analysis was carried out on cDNA prepared from the isometamidium sensitive trypanosome clone, IL1180, and its resistant derivative, IL1180x200R. The resultant polymorphic bands were cloned, sequenced and homology searches performed on them. Two of these RAPD bands showing significant homology with proteasome and metallothionein-A revealed restriction fragment length polymorphisms when hybridised to blots containing digests of genomic DNA prepared from IL1180 and its isometamidium resistant derivatives. These RAPD bands were used to screen cDNA libraries prepared from the isogenic trypanosome clones, to obtain full-length transcripts of the corresponding genes. Nucleotide sequences of the proteasome gene transcripts so obtained were determined and analysed for polymorphisms. The sequences were then translated into amino acid sequences and compared to determine if any of the nucleotide sequence differences resulted in changes in the deduced amino acid sequences.

Several polymorphisms in nucleotide sequences between the proteasome gene transcripts obtained from the cDNA libraries of the isometamidium sensitive and resistant trypanosome clones were detected. Those differences that occur within the open reading frame are in position 302, 335, 385, 662 and 663. Two of these polymorphisms which occurred within the translated region at positions 385 and 662 resulted in changes at the amino acid level. These were

the substitution of valine for alanine and serine for threonine respectively. Several mutations also occur within the 5' untranslated region (UTR), and three in the 3' UTR. In most cases the differences observed are single base changes, though in two instances, such as in positions 663 (change of TA to AT) and 1104 (insertion of GG), the nucleotide sequence differences are in pairs.