8 SUMMARY

Resistance to isometamidium is a serious problem in many parts of sub-Saharan Africa. Several tests have been described for the detection of drug resistance in trypanosomes, but the techniques currently used suffer from a number of drawbacks. Therefore, faster, more sensitive and more reliable methods are required. The main objectives of the current studies were a) assessing the isometamidium sensitivities of *Trypanosoma congolense* clones derived from trypanosome stocks from naturally infected cattle in East and West Africa, b) inducing isometamidium resistance in a drug sensitive *T. congolense* clone, and c) characterising the putatively resistance-related nucleoside transporter gene (TbAT1) in isometamidium-sensitive and -resistant *T. brucei brucei* and *T. congolense*.

Trypanosome clones were derived from *T. congolense* stocks collected from cattle in Ethiopia and Burkina Faso with different isometamidium sensitivity phenotypes. An overall cloning success rate of 20.30% was achieved, which ranged from 16.67 to 25.00% for the different stocks. The isometamidium sensitivities of the clones were assessed to multiple dosages of isometamidium in mice. The results demonstrated that all the *T. congolense* clones expressed high levels of resistance to isometamidium when compared to known isometamidium-sensitive *T. congolense* reference clones. The clones from Burkina Faso expressed significantly ($p<0.05$) higher levels of resistance in mice than the clones from Ethiopia. Analyses of variances of the mean relapse intervals showed that for most of the clones tested there were clear relationships between the time of relapses and the doses of isometamidium used; mice treated with lower doses relapsed after a shorter time than mice treated with higher doses ($p=0.001$). The CD_{50} values for the clones from Ethiopia ranged from 9.86 to 13.37 mg/kg bw, whereas, the clones from Burkina Faso had CD_{50} values ranging from 19.80 to > 20.0 mg/kg bw. There was no significant ($p>0.05$) variation in expression of resistance to isometamidium among three of the clones derived from a single stock (PA 77) from Ethiopia. Similarly, two of the clones derived from a stock of Burkina Faso (SA 95) expressed a similar ($p>0.05$) level of resistance to isometamidium.

With the aim of understanding the developmental mechanism of resistance in trypanosomes, clones of *T. congolense* with different level of resistance to isometamidium were derived from a drug sensitive clone in mice. The levels of resistance of IL 2642 clone to isometamidium was increased at least 159-fold (from a CD_{50} of 0.0086 to 1.37 mg/kg bw) by repeated subcurative treatment of infected mice over a period of 16 months. This was associated with 2.2-fold increase (from a CD_{50} of 8.20 to 18.02 mg/kg bw) in resistance to diminazene aceturate, tested in mice. The results indicate that administering drugs below effective levels can produce drug resistance. The high level of resistance developed in immunosuppressed mice in the current study was stable and persisted when the clones were subsequently tested

in immunocompetent mice. However, similar drug doses and similar protocols in normal immunocompetent mice failed to lead to development of isometamidium resistance in the IL 2642 clone. This shows that immunosuppression of the host may considerably reduce the efficacy of trypanocidal drugs and can lead to the rapid development of drug resistance.

Analyses were made on the TbAT1 gene, which encodes for the P2 transporter, from *T. b. brucei* field stocks to investigate a possible link between the presence of mutations in this gene and isometamidium resistance. We have analysed the TbAT1 gene of *T. b. brucei* from 11 isometamidium-sensitive field stocks, two sensitive reference clones and two resistant reference clones. Sequence alignment showed that the isometamidium-sensitive *T. b. brucei* contained the wild-type sequence patterns. In contrast, the isometamidium-resistant *T. b. brucei* stocks showed the mutant-type sequence patterns that corresponded to the DNA sequence of the laboratory-derived melarsoprol-resistant STIB 777R stock. Six point mutations were detected in the isometamidium-resistant stocks, which were also described earlier in the laboratory-derived melarsoprol-resistant stock of *T. brucei*. Four of these nucleotide differences detected lead to amino acid substitutions: $Ala^{178} \rightarrow$ Thr (A178T), $Gly^{181} \rightarrow$ Glu (Gl181E), $Asp^{239} \rightarrow$ Gly (D239G) and $Asn^{286} \rightarrow$ Ser (N286S). Furthermore, deletions of three nucleotides (TTC) that encode the amino acid phenylalanine were detected in the resistant stocks. The point mutation that led to the substitution of G by A at nucleotide position 532 (G532A) in the sensitive stock eliminated the Sfa NI restriction site. Whereas, the mutation at 857 bp that led to the substitution of A by G (A857G) generated a new Sfa NI restriction site. Consequently, in order to analyse the RFLP pattern of a fragment of TbAT1 (nucleotides 430-1108), the 677 bp PCR products from eight of the isometamidium-sensitive and two of the isometamidium-resistant *T. b. brucei* were subjected to digestion with Sfa NI. The results revealed two different banding patterns: the digest produced fragment sizes of 566 and 111 bp in the case of TbAT1 from isometamidium-sensitive stocks, whereas it produced fragment sizes of 435 and 242 bp in the case of TbAT1 from isometamidium-resistant stocks. Thus, the isometamidium-sensitive and resistant *T. b. brucei* could be successfully distinguished by digestion with the restriction endonuclease Sfa NI. It can therefore be concluded that there is a link between the presence of mutations in the nucleotide transporter gene (TbAT1) in *T. b. brucei* and isometamidium resistance. Furthermore, Sfa NI-RFLP, if validated with a large scale screening of field isolates, may serve as a convenient diagnostic tool for rapid identification of isometamidium-resistant *T. b. brucei*. The attempts made to amplify the TbAT1 homologous gene from the genomic DNA of *T. congolense* failed.