

In vitro efficacy of anti-protozoal compounds as a novel treatment for *Tritrichomonas foetus* in bulls

J.H. Koziol, DVM, DACT¹; D. F. Wolfe, DVM, MS, DACT¹; M.A. Edmondson, DVM, MS, DACT¹; S.H. Duran, RPh, MS, PhD¹; T. Passler, DVM, PhD, DACVIM^{1,2}; J.A. Gard, DVM, PhD, DACT¹

¹Department of Clinical Sciences, Auburn University, Auburn, Alabama 36849

²Department of Pathobiology, Auburn University, Auburn, Alabama 36849

Introduction

Currently, there are no legal treatments in the United States for cattle infected with *Tritrichomonas foetus*. This obligate parasite of the reproductive tract of the bovid creates serious economic loss in the cattle industry in the United States. The hypothesis of this study was that benzimidazoles (oxibendazole or oxfendazole) or ponazuril combined with a polymer enhancer, specifically polymer lecithin organogel (PLO), in a topical formulation may be an effective treatment for bulls infected with *T. foetus*. Three *in vitro* experiments were performed to evaluate the antiprotozoal effects of the benzimidazole and ponazuril formulations, and all involved components on the growth of *T. foetus* organisms.

Materials and Methods

Three *in vitro* experiments were performed using pure cultures of *T. foetus* trophozoites with the addition of the drugs alone or the polymer-enhanced drugs to either culture tubes or tissue culture wells. Samples were evaluated at predetermined time points. Neubauer hemocytometers were used to quantify the number of viable organisms and formation of pseudocysts. Pseudocysts were characterized by the rounding of the cell with internalization of the flagella. Incubation was continued for all cultures in which non-motile trophozoites or pseudocysts were identified to allow for reversibility of the organism to the motile trophozoite form. In Experiment I, oxibendazole (OX) or ponazuril (PO) alone or combined with PLO were tested for *in vitro* efficacy. In Experiment II, to increase the concentration of the benzimidazole, oxfendazole (OXF) powder was reconstituted to a solution with 70% ethanol (EtOH) and mixed with Velvachol (VC) prior to addition of PLO. The OXF solution (OXF/EtOH/VC/PLO) was evaluated for its ability to inhibit growth of trophozoites. Experiment III evaluated each of the 4 components (OXF, VC, EtOH, and PLO) in all possible combinations for inhibition of *T. foetus* to determine which components of the solution used in Experiment II were responsible for inducing the pseudocyst

form and leading to demise of the organism. Evaluation of the inhibitory potential of EtOH and dimethyl sulfoxide (DMSO) was also included in Experiment III.

Results

All antiprotozoals tested inhibited the *in vitro* growth of *T. foetus*. In Experiment I, the inhibitory effects of PO and OX in combination with PLO significantly inhibited parasite growth at concentrations of 75 mg/mL⁻¹ and 50 mg/mL⁻¹, respectively. Each respective culture was negative for motile trophozoites at 24 hours post-treatment. However, motile organisms were once again present at 48 hours. In Experiment II the formulation in which the concentration of benzimidazole in culture was increased to 150 mg/mL⁻¹ by the use of OXF/EtOH/VC with PLO and was found to have substantial inhibitory effects on trichomonad growth. The pseudocyst or endoflagellar state was induced with no motile trichomonads detected in the culture following an incubation period of 4 hours. The pseudocyst state persisted for 24 hours and reversibility to trophozoites was not detected. Experiment III revealed that the OXF solution, when combined with PLO and EtOH alone, rapidly induced the pseudocyst stage and led to complete disappearance of organisms following culture.

Significance

This series of studies demonstrated several interesting findings. First, benzimidazoles and ponazuril can negatively impact the growth of the *T. foetus* organism in culture. Second, that PLO can be used in combination with benzimidazoles and ponazuril without negatively impacting the effect of the antiprotozoals on *T. foetus*. Third, that oxfendazole combined with PLO gel can inhibit the growth and lead to complete kill of the bovine strain CDTf3 of *Tritrichomonas foetus* in *in vitro* cultures. Lastly, a combination of oxfendazole and PLO gels exhibits efficacy similar to 70% ethanol, which is commonly used to destroy the organism in the laboratory.