ABSTRACT

In vitro antimicrobial susceptibility testing was used to compare the efficacy of imidocarb dipropionate and doxycycline on the growth of Ehrlichia canis in DH82 cell cultures. Over a 9-day period there were no significant differences (p < 0.01) in the growth of E. canis in untreated control wells and those to which imidocarb dipropionate was added at 1.2, 2.4, 4.8 or 12 µg/ml for the 1st 3 days. Average infection rates rose from 50 to 55 % on day 0 to 100 % on day 5 or 6. Doxycycline at 1 µg/ml had residual or rickettsiocidal activity against E. canis with the average percentages of DH82 cells infected declining from 51 to 24 % while the organism was exposed to the drug (3 days) and from 21 to 2 % in the 6 days following removal of the drug from the cell culture medium.

Key words: Ehrlichia canis, doxycycline, imidocarb dipropionate, sensitivity testing.


INTRODUCTION

Dogs in southern Africa are commonly infected with Ehrlichia canis, the agent of canine ehrlichiosis8,9,11. Self-cure is not known to occur and dogs remain infected until the organisms are cleared from the animal by appropriate therapy27,14. While in vitro studies have shown chloramphenicol, procaine penicillin G, sulfadimethoxine and sulfacetamide to be ineffective in the treatment of canine ehrlichiosis8, tetracycline therapy has been shown to be highly effective27,34. Treatment with tetracycline has, however, been reported to have numerous drawbacks such as vomiting, lack of owner compliance with the longterm therapy required and staining of dental enamel in young animals31.

The use of imidocarb dipropionate, a drug used widely in Africa for the treatment of canine babesiosis, has been reported to have fewer drawbacks. The efficacy of the drug in the treatment of canine ehrlichiosis has been described13,8,21,11,24. In these reports the treatment regimens varied considerably and it is unclear whether the efficacy of imidocarb dipropionate results from high plasma concentrations of the drug for a short time or lower concentrations maintained for longer periods23. Since Ehrlichia spp. are obligate intracellular organisms it has not been possible to determine the efficacy of antimicrobials on these bacteria using conventional susceptibility tests. Recently, however, assay systems have been developed to evaluate the susceptibilities of intracellular organisms to antimicrobials in vitro4,5,12. In this report we describe the results of experiments using these new methods to compare the effects of doxycycline and imidocarb dipropionate on E. canis.

MATERIALS AND METHODS

E. canis (Oklahoma strain) was grown in DH82 cells34 in 175 cm² tissue culture flasks using minimal essential medium supplemented with 12.5 % foetal bovine serum and 2 mM L-glutamine. When cells gently scraped from the flask and stained with Diff-Quick and the ratio of infected cells compared to non-treated controls indicated that the drug had a residual or rickettsiocidal effect on E. canis. These results are consistent with a previous report on in vitro antimicrobial sensitivity testing against E. canis15 in which doxycycline and rifampacin were shown to have rickettsiodial activity, while penicillin, gentamycin, co-trimoxazole, chloramphonic, pefloxacin and erythromycin were found to be ineffective. The results are also consistent with reports that tetracycline therapy is effective in the treatment of canine ehrlichiosis in vivo26,17.

The concentrations of imidocarb dipropionate used in our experiments (1.2, 2.4, 4.8 and 12 µg/ml) were empirical as there are no data on the pharmacokinetics of imidocarb dipropionate in dogs following intramuscular or subcutaneous administration1. In sheep, the only species for which such data are available, an intramuscular injection of 4.5 mg/kg results in blood drug levels of about 4.5 µg/ml for 5 d. This subsequently decreases slowly by first-order kinetics, reaching concentrations of less than 1 µg/ml by day 28. Similar doses of imidocarb dipropionate (5–7 mg/kg) administered subcutaneously22 or intra-
E. canis infections with imidocarb dipropionate results from prolonged exposure of the organism to the drug. We would note, however, that previous reports of the efficacy of imidocarb dipropionate on E. canis can also be explained by self-cure and circumstantial evidence has been presented that this might occur. Further studies in which the possibility of self-cure in dogs with canine ehrlichiosis is assessed and also the effects of various treatment regimens with imidocarb dipropionate are required to finally resolve the question of the therapeutic efficacy of the drug.

**ACKNOWLEDGEMENTS**

Funding was provided by the Research Board of the University of Zimbabwe and the European Community-sponsored link between the Veterinary Faculties of the Universities of Zimbabwe and Utrecht.

**REFERENCES**