Lack of susceptibility of *Ehrlichia canis* to imidocarb dipropionate *in vitro*

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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.

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INTRODUCTION

Dogs in southern Africa are commonly infected with *Ehrlichia canis,* the agent of canine ehrlichiosis[8,9,11]. Self-cure is not known to occur and dogs remain infected until the organisms are cleared from the animal by appropriate therapy[3,7,14]. While *in vivo* studies have shown chloramphenicol, procaine penicillin G, sulfadimethoxine and sulfacetamide to be ineffective in the treatment of canine ehrlichiosis[6], tetracycline therapy has been shown to be highly effective[3,7,16]. 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 animals[13].

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 described[1,10,12,13]. 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 periods[10]. 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 vitro*[4,5,15]. 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 cells[18] in 175 cm^2 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 (American Scientific Products, Obetz, Ohio) were found to be 30–50 % infected, the remaining cells were harvested using phosphate-buffered saline (pH 7.2) with 0.2 % EDTA. After addition of 15 mℓ fresh medium, the cell suspension was transferred to 96 well tissue culture plates (200 µℓ per well) and incubated at 37 °C until the cells became confluent. The medium was removed and each of the middle 6 rows in the plate were filled with 100 µℓ antimicrobial-free medium (controls) or medium containing 1 µg/mℓ doxycycline or 1.2, 2.4, 4.8 or 12 µg/mℓ imidocarb dipropionate (Imizol, Coopers Animal Health, England). The cells were incubated for 3 d before the medium was removed and replaced with fresh antimicrobial-free medium. Cells were harvested from one well in each row on days 1–9 after the addition of antimicrobial-containing medium and centrifuged onto glass slides using a cytospin at 600 g. After air-drying, the cells were stained with Diff-Quick and the ratio of infected to non-infected cells was independently determined by at least 2 of the authors with a Leitz microscope at ×400 magnification. All experiments were performed in triplicate and Student's *t*-test used to analyse data.

RESULTS

The addition of doxycycline (1 µg/mℓ) to DH82 cells infected with *E. canis* resulted in a decrease in the proportion of infected cells compared to non-treated controls (Fig. 1). Following the removal of doxycycline from the cell culture medium, the proportion of infected cells continued to decline, indicating 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. canis*[4] in which doxycycline and rifampacin were shown to have rickettsiocidal activity, while penicillin, gentamycin, co-trimoxazole, chloramphenicol, 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 vivo*[3,7,16,17].

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 administration[10]. 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[2]. Similar doses of imidocarb dipropionate (5–7 mg/kg) administered subcutaneously[12] or intra-

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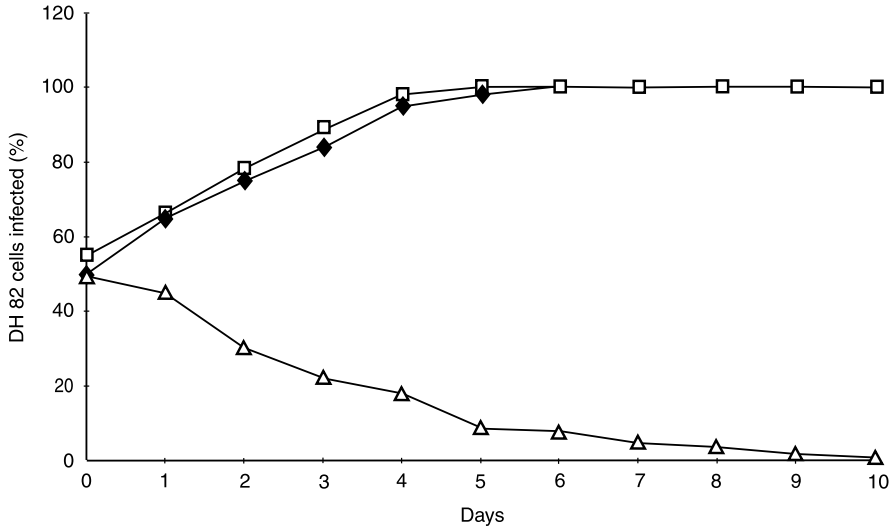

Fig. 1: **Average percentages of DH82 cells infected with *E. canis* in wells harvested daily from untreated rows (❑) and those treated for 3 days with doxycycline at 1 μg/mℓ (Δ) or imidocarb dipropionate at 1.2, 2.4, 4.8 and 12 μg/mℓ (◆).**

muscularly[1] have been reported to be effective in the treatment of canine ehrlichiosis.

On each day of our experiments there were very similar percentages of infected cells in the control wells and those to which imidocarb dipropionate was added at 1.2, 2.4, 4.8 or 12 μg/mℓ (results not shown). The differences between the percentage of infected cells in individual wells and the averages for the control and imidocarb dipropionate-treated wells on each day did not exceed 8 % (results not given). Similarly, there were no significant differences ($p < 0.01$) between the averages of the percentages of infected cells in wells treated with the different concentrations of imidocarb dipropionate and those of the untreated controls for the duration of the experiment (Fig. 1).

DISCUSSION

Our findings indicate that the *in vitro* growth of *E. canis* is unaffected by short-term (3 days) exposure to apparently low or high levels of imidocarb dipropionate. It therefore seems unlikely that the reported efficacy of imidocarb dipropionate against *E. canis* results from high plasma concentrations maintained for a short time. This is consistent with results of previous experiments[17] in which clinical ehrlichiosis was observed in dogs inoculated with blood from infected dogs treated with imidocarb dipropionate 14 days previously. It therefore appears likely that the successful treatment of *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[10] and circumstantial evidence has been presented that this might occur[11]. 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.

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