

Prednisolone at Anti-Inflammatory or Immunosuppressive Dosages in Conjunction with Doxycycline Does Not Potentiate the Severity of *Rickettsia rickettsii* Infection in Dogs

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Dogs were experimentally inoculated with *Rickettsia rickettsii* to determine if anti-inflammatory or immunosuppressive dosages of prednisolone, when administered in conjunction with an antirickettsial antibiotic (doxycycline), induce therapeutically relevant pathophysiologic consequences that ultimately influence disease outcome. Although the duration of rickettsemia was prolonged in dogs receiving immunosuppressive, but not anti-inflammatory, corticosteroids, concurrent administration of doxycycline and corticosteroids conferred no other detected detrimental effects. Treatment with doxycycline or doxycycline in conjunction with prednisolone resulted in decreased *R. rickettsii*-specific antibody titers; however, examination of appropriately timed acute- and convalescent-phase serum samples would have facilitated an accurate diagnosis of Rocky Mountain spotted fever (RMSF) in all 16 dogs. We conclude that the concurrent use of anti-inflammatory or immunosuppressive doses of prednisolone in conjunction with doxycycline, early in the course of experimental RMSF, confers no clinically relevant detrimental effects and that additional studies might be indicated to detect possible beneficial effects in cases of severe or potentially fulminant RMSF. However, because the illness induced in these dogs was of mild to moderate severity, the results of this study should definitely not be construed as supporting the safety or efficacy of prednisolone for treatment of severe canine or human RMSF.

Rocky Mountain spotted fever (RMSF), caused by *Rickettsia rickettsii*, is the most common human rickettsiosis in the United States. Although definitive incidence data are not available, RMSF is a common cause of acute, potentially fatal, febrile illness in dogs, particularly in the southern and middle eastern United States. Previous research from our laboratory involving studies of experimental *R. rickettsii* infection in dogs (1-3, 6, 7, 9) has helped to clarify selected clinical, hematologic, serologic, and therapeutic questions related to natural infection. In conjunction with these studies, we have shown that the pathophysiologic consequences of cytopathic rickettsial vascular injury in dogs are very similar to changes described for similarly infected humans (19). Due to inaccurate or delayed diagnoses, as well as other undetermined factors, mortality associated with human RMSF has not decreased in the postantibiotic era (8). As with the disease in humans, canine RMSF is often associated with severe morbidity and occasional mortality. Because of the disease similarities, information generated through experimental studies utilizing dogs may be applicable to both species.

The use of corticosteroids as adjunctive treatment for rickettsial diseases is controversial, and definitive data to substantiate beneficial or detrimental effects are lacking. Anti-inflammatory doses of corticosteroids are frequently used for treatment of the ocular manifestations of RMSF, which in dogs include anterior uveitis, ocular hemorrhage, and exudative retinopathy (5). Because acute RMSF can be accompanied by a rapid drop in erythrocyte and thrombocyte numbers, physicians and veterinarians can be confronted with the dilemma of initiating immunosuppressive corticosteroid treatment in a

critically ill, febrile patient or dog, for which the tentative diagnoses include immunologically mediated anemia and/or immunity-mediated thrombocytopenia or severe rickettsial disease. Based upon the development of gangrenous necrosis of the extremities in some *R. rickettsii*-infected dogs treated with immunosuppressive dosages of corticosteroids and subsequently referred to the North Carolina State University Veterinary Teaching Hospital, our clinical experience suggested that concurrent corticosteroid treatment might induce mortality or potentiate the severity of RMSF. Because numerous other factors may have influenced the clinical course of RMSF in naturally infected dogs, we attempted to determine if there are therapeutically relevant pathophysiologic consequences associated with concurrent doxycycline and prednisolone treatment in dogs experimentally infected with *R. rickettsii*.

MATERIALS AND METHODS

Study design. Sixteen 13-month-old female beagles, which had received vaccinations for canine distemper, adenovirus, parvovirus, parainfluenza virus, coronavirus, and rabies virus and monthly heartworm prophylaxis, were obtained from Intervet Inc. (Millsboro, Del.). The dogs were randomly assigned to four groups (nontreated infection control, doxycycline treated, doxycycline-low-dose prednisolone treated, doxycycline-high-dose prednisolone treated), with four dogs in each group. During the study, the dogs were cared for in accordance with P-3 biosafety level isolation procedures. All dogs were seronegative to *R. rickettsii* by microimmunofluorescence testing as described previously (1) and were negative for dirofilariasis and intestinal parasites. Complete blood counts were normal prior to infection.

Inoculum. All four groups were inoculated with *R. rickettsii* (NCSU strain Domino), a canine isolate (inoculum strength, 5×10^5 PFU/ml), intradermally in two sites over the pelvis (0.5 ml/site). Rickettsiae were diluted in sterile brain heart infusion broth for inoculation. Previous studies from our laboratory have established that this inoculum strength would be expected to produce moderately severe nonfatal infection in dogs (1-3, 6, 9).

Treatment protocol. Beginning on postinoculation day (PID) 7 and continuing for 7 consecutive days, doxycycline hyclate tablets (Schein Pharmaceutical Inc., Florham Park, N.J.) or doxycycline hyclate and prednisolone tablets (Danbury

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Pharmaceutical Inc., Danbury, Conn.) were given to four dogs in each of the respective treatment groups. Because of variations in body weight, the dosage of drug administered differed slightly among dogs within a group. The mean body weight of the 12 dogs in the three treatment groups was 9.9 kg, with a range from 7.1 to 13.6 kg. The mean doxycycline dosage for the 12 dogs was 5.1 mg/kg of body weight given orally at 12-h intervals (total daily dose, 10.2 mg/kg). The mean anti-inflammatory prednisolone dose was 0.57 mg/kg given orally once daily in the morning, and the mean immunosuppressive prednisolone dose was 2.0 mg/kg given orally at 12-h intervals. In all three treatment groups, drug administration was discontinued after 7 consecutive days of administration.

Clinicopathologic testing. Physical examinations, including rectal temperature measurements, observations related to attitudinal change, and oral and ocular examinations, were conducted in an examiner-blinded fashion twice daily. An attitudinal score was recorded for each dog twice daily by using the following criteria: 5, alert, active, and eating; 4, alert, inactive, and eating; 3, depressed, inactive, and anorectic; 2, severely depressed and anorectic; 1, recumbent; and 0, dead. Blood was collected by jugular venipuncture on PID 3, 5, 7, 10, 12, 14, 18, and 21 for complete blood counts (Clinical Pathology Laboratory, North Carolina State University Veterinary Teaching Hospital), selected serum biochemical analyses (VetTest 8008, IDEXX Laboratories Inc., Westbrook, Maine), and serologic testing for *R. rickettsii* by microimmunofluorescence (1, 2).

Vascular permeability studies. Fluorescein angiography of the ocular fundus was performed prior to inoculation and on PID 6, 10, and 17 by previously described methods (6). Sites of sodium fluorescein (NaFl) dye leakage from injured retinal vessels were quantitated visually.

Doxycycline analysis in plasma. Plasma for doxycycline analysis was collected from treated dogs on the first day of doxycycline administration (PID 7) and again on administration day 7 (PID 14). Blood samples were obtained by jugular venipuncture at 0, 30, 60, 180, 300, and 720 min after doxycycline administration. Doxycycline in plasma was measured by high-performance liquid chromatography (HPLC). The method used was modified from the method reported by Riond and Riviere (16).

Analysis of plasma doxycycline concentration profile. Plasma drug concentration versus time curves for each dog were plotted on semilogarithmic graphs. The terminal portion of the graphs was analyzed by simple linear regression to determine the slope and intercept of the curve. The slope represents the rate constant (k) for the elimination of doxycycline from which the half-life was calculated as follows: half-life (h) = 0.693/ k . The intercept (C_0) represents the plasma drug concentration curve extrapolated to time zero. For some of the plasma drug concentration profiles, it was not possible to calculate a terminal elimination rate and half-life because after the absorption phase of the curve, there were not enough points to accurately determine a straight line.

The area-under-the-curve (AUC) for the plasma drug concentration versus time from 30 min to 8 h was calculated using the trapezoidal rule described by Rowland and Tozer (18). The maximal plasma drug concentration during the dosing interval (C_{max}) was taken directly from the plasma drug concentration-time curve.

Rickettsial isolation. Venous blood, collected aseptically from the jugular veins of all 16 dogs on PID 6 and 10, was used to inoculate Vero cell cultures by two protocols. First, 0.1 ml of whole heparinized blood was overlaid on confluent monolayers in 25-cm² flasks. After adsorbing for 2 h, cultures were fed with medium 199 (Gibco, Grand Island, N.Y.) containing 7.5% fetal bovine serum (Hyclone) and incubated at 35°C with 5% CO₂. On PID 10, monolayers were resuspended and slides were made from cell suspensions in phosphate-buffered saline. After air drying and acetone fixation, slides were stained by Gimenez stain and by direct fluorescence by using fluorescein-labeled rabbit anti-*R. rickettsii* (Centers for Disease Control, Atlanta, Ga.) and examined by light and UV microscopes, respectively. In the second protocol, 10-fold dilutions of whole blood (10⁰ to 10³) were placed in shell vials, each containing a coverslip with a confluent monolayer of Vero cells (12). Vials were centrifuged at 700 × g for 1 h prior to incubation at 35°C with 5% CO₂. At 48, 72, and 96 h for cultures begun on PID 6 and at 72, 96, and 120 h for cultures begun on PID 10, coverslips were acetone fixed and stored at -20°C until stained by direct fluorescence and examined at 40× with a UV microscope.

Microimmunofluorescence testing. Microimmunofluorescence testing was used to determine the presence of antibodies to *R. rickettsii* (strain Domino). Dog sera were examined by using fluorescein isothiocyanate goat anti-canine immunoglobulin G (heavy and light chain-specific) conjugate. All sera were titrated to end point. Positive and negative controls were evaluated with each group.

Statistics. Treatment effects were analyzed by using multivariate repeated-measures analysis of variance (ANOVA) (10) using PROC GLM software from SAS (version 6-09) (SAS Inc., Cary, N.C.). A separate analysis was performed for each physiological parameter. A Student-Neuman-Kauls comparison procedure at an alpha of 0.05 was carried out at each time to differentiate significant effects among the treatments. The analysis was done separately for times prior to treatment and for times after treatments. Profile contrasts were used to detect significant differences in the shape of the parameter response curves among the treatment groups. The nonparametric Kruskal Wallis test was performed on attitudinal scores and the retinal vascular lesion data due to the non-normality of the data. Proc NPARIWAY software from SAS (version 6.09) was used.

The pharmacokinetic parameters (AUC and C_{max}) were compared by a two-

way ANOVA to determine if there were differences in doxycycline disposition related to duration of treatment (first dose versus 10th dose) or related to adjunctive therapy with corticosteroids. A SAS computer program was used for analysis.

RESULTS

Clinicopathologic studies. Prior to the initiation of the treatments, there were no significant differences (P) in mean values for rectal temperature ($P = 0.28$), attitudinal score ($P = 0.5$), neutrophil number ($P = 0.39$), platelet number ($P = 0.40$), or the percent serum albumin/serum total protein concentration ($P = 0.31$) among the four groups of dogs. As in a previous study from our laboratory (1), fever (rectal temperature $\geq 39.6^\circ\text{C}$) was the first indication of infection. On PID 3, all dogs had at least one rectal temperature measurement of $>40^\circ\text{C}$. Through PID 6, all 16 dogs remained febrile. However, on the morning of PID 7, prior to the beginning of treatments, two dogs (both in the infection control group) were no longer febrile. Between PID 7 and 10 there was a trend in mean rectal temperature measurements (infection control $>$ doxycycline $>$ doxycycline-anti-inflammatory prednisolone $>$ doxycycline-immunosuppressive prednisolone) among the groups. Deferescence occurred in all four infection control dogs by PID 8; however, statistically significant decreases in rectal temperature were detected for each of the treatment groups compared to the infection control group (Wilks lambda, $P = 0.006$, 0.01, and 0.0009, respectively). Beginning with the p.m. temperature measurements on PID 7 and persisting with a.m. and p.m. temperature measurements through PID 9, there was a decrease in rectal temperature in both the anti-inflammatory and immunosuppressive prednisolone groups compared to the infection control group. By multiple comparison, the mean p.m. rectal temperature measurements on PID 7 of dogs receiving immunosuppressive prednisolone and doxycycline were lower than mean temperatures for the other three groups.

Physical examination abnormalities were not noted until PID 4, at which time most of the dogs developed mucopurulent ocular discharge, episcleral injection, and aural hyperemia. By PID 5, clear discrete vesicles that subsequently progressed to pustules which ruptured and healed during the next several days were observed on the oral mucous membranes of most dogs. Attitudinal scores decreased from 5 to 4 in all dogs between PID 5 and 6. Treatments were initiated within 24 h after attitudinal scores began to decrease. Daily attitudinal scores ranged from 5 to 3 in all groups throughout the study (Fig. 1). Compared to the infection control group, treatment groups experienced a statistically significant improvement in attitudinal score ($P = 0.001$) by 48 h after initiation of treatment.

Thrombocytopenia, decreased albumin/total protein ratios, and visual quantitation of retinal vascular lesions were used to compare the degree of rickettsially induced vascular injury. Overall treatment effects produced platelet numbers different ($P = 0.005$) from those for the control group. By using multiple comparisons, it was shown that all three treatment groups had higher mean platelet counts on PID 10 and 13 (Fig. 2). On PID 13, all four groups differed from each other, but by PID 18 mean platelet counts were not significantly different among the four groups. Similarly, the doxycycline-anti-inflammatory and doxycycline-immunosuppressive prednisolone groups experienced a significant decrease in albumin loss on PID 13 and 18 compared to the doxycycline or infection control groups (Fig. 3). Prior to initiation of therapy, there were no differences in the total number of retinal vascular lesions among the four groups as detected by fluorescein angiography. Although there was a decrease in retinal lesions in all treatment groups, pre-

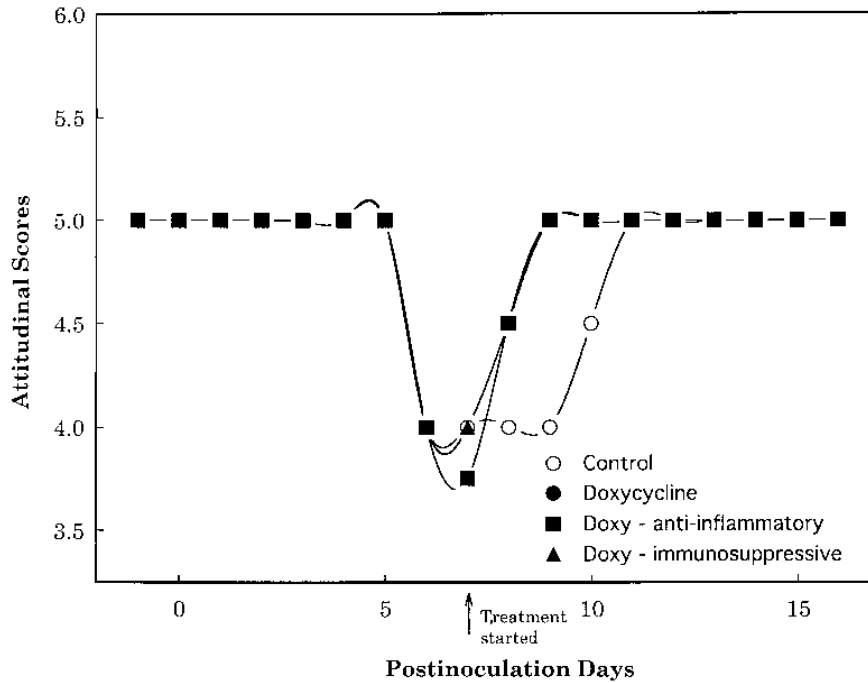


FIG. 1. Mean daily attitudinal scores after inoculation of *R. rickettsii* (day 0) and after initiation of treatments (day 7).

sumably associated with more rapid therapeutic improvement in vascular integrity, this result was not significant, most likely due to the low number of retinal vascular lesions observed in this study (Fig. 4). Subjectively there was more rapid resolution of periocular hyperemia and episcleral injection in the prednisolone treatment groups, particularly the immunosuppressive group. Although overall treatment effects on mean absolute neutrophil numbers made these numbers significantly different from those for the control group ($P = 0.03$), the biological significance of these data is doubtful. Following initiation of treatment (PID 10 and 13), the doxycycline and doxycycline-anti-inflammatory prednisolone groups had decreased mean neutrophil counts, whereas the doxycycline-im-

munosuppressive prednisolone group had neutrophil counts that were higher than or similar to those for the control group.

Doxycycline plasma profile. The concentration of doxycycline in plasma is shown on Fig. 5 (trials 1 and 2). Table 1 provides the pharmacokinetic values calculated for each trial and treatment. Trial 1 is on the first day of treatment (PID 7), and trial 2 followed doxycycline administration on the seventh day of treatment (PID 14). Treatment (doxycycline alone, doxycycline-anti-inflammatory prednisolone, and doxycycline-immunosuppressive dose prednisolone) had no significant effect on the values of AUC and C_{max} , irrespective of whether the treatment occurred in trial 1 or trial 2 (i.e., after the initial dose or after the 10th dose). The duration of doxycycline

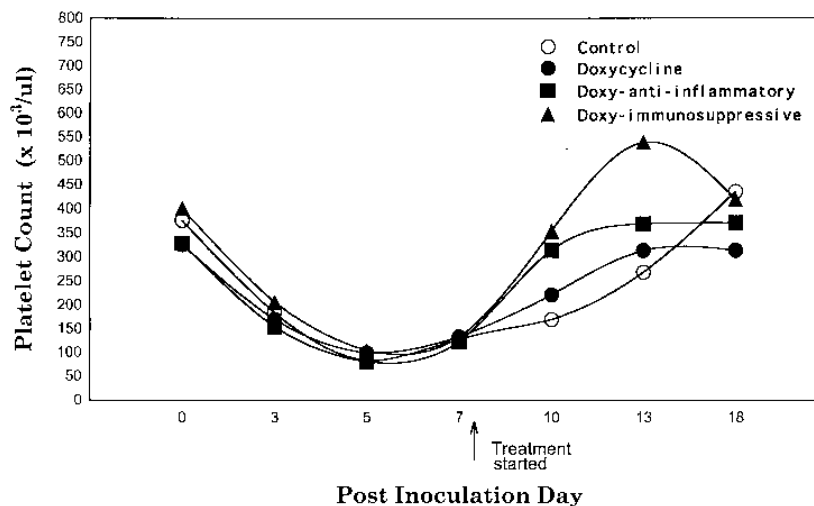


FIG. 2. Mean platelet values of the infection control and treatment groups after inoculation with *R. rickettsii* (day 0) and after the initiation of treatments (day 7).

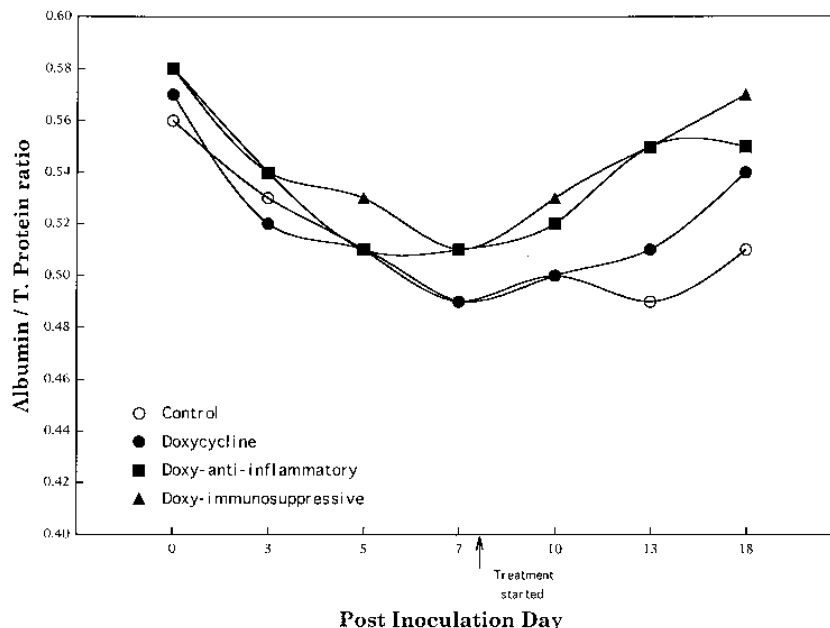


FIG. 3. Mean ratios of albumin to total protein for the infection control and the treatment groups after inoculation with *R. rickettsii* (day 0) and after initiation of treatments.

administration had no statistical effect on the plasma profile (i.e., no significant interaction between treatment and trial).

As expected, there was a difference in C_{max} and AUC between trial 1 and trial 2. Because the dose interval is shorter than $5 \times$ the half-life, doxycycline will accumulate in dogs with repeated dosing every 12 h. After the 10th dose, the AUC and C_{max} were significantly higher in trial 2 than in trial 1 ($P = 0.0003$ and 0.0013 , respectively).

Rickettsiae isolation. The attempt to isolate rickettsiae in 25-cm² flasks resulted in extensive contamination in the cultures begun on PID 6. Therefore, no results could be obtained.

The shell vial attempt begun on PID 6 gave negative results before 96 h. At 96 h, 11 of 16 cultures were positive at 10^0 , of which 3 remained positive at 10^1 . Positive cultures were obtained from three of four dogs in the infection control, doxycycline, and doxycycline-immunosuppressive prednisolone groups and from two of four dogs in the doxycycline-anti-inflammatory prednisolone group.

The attempt to isolate in flasks blood collected on PID 10 resulted in cultures in which no rickettsial organisms were detectable by Gimenez stain or direct immunofluorescence. Shell vial cultures from PID 10, harvested at 120 h postinocu-

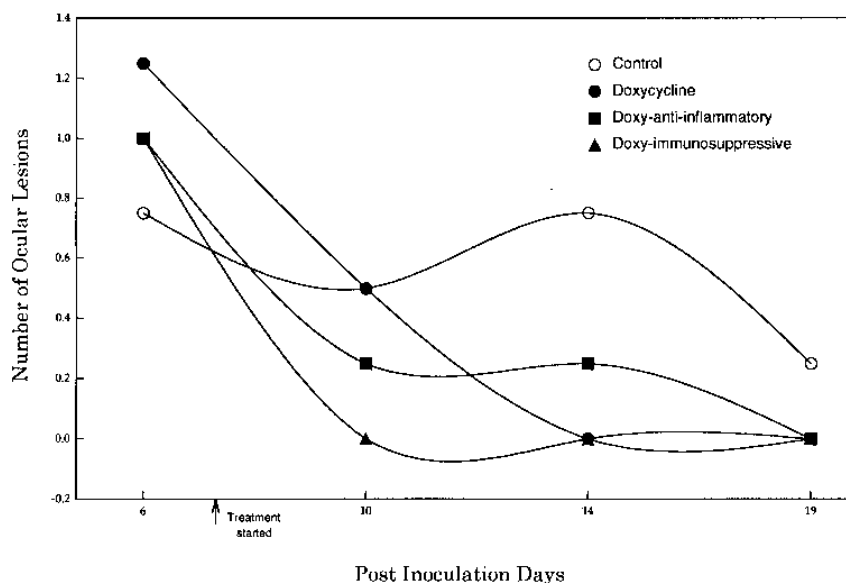


FIG. 4. Mean number of retinal vascular lesions detected by fluorescein angiography in the control and treatment groups after inoculation with *R. rickettsii* (day 0) and after the initiation of treatments (day 7).

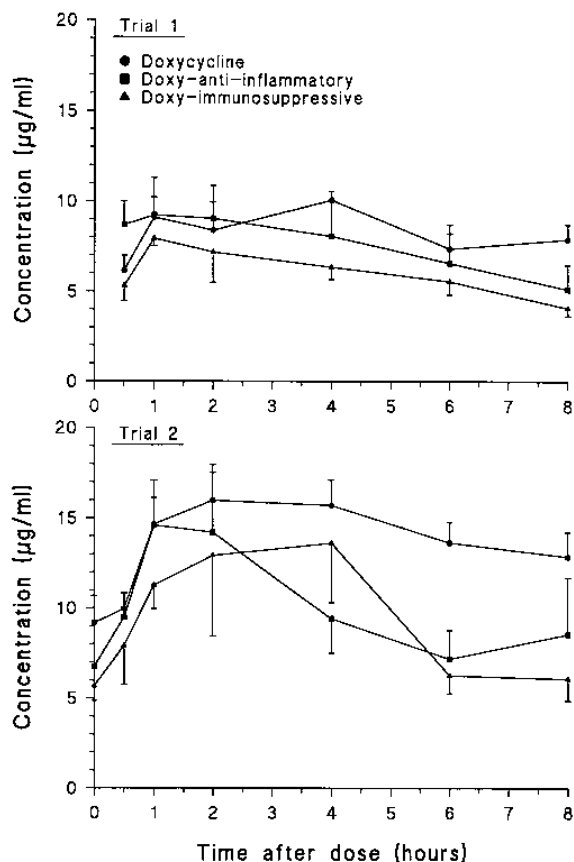


FIG. 5. Doxycycline concentrations in the three treatment groups following the initial dose (trial 1) and following the 10th drug dose (trial 2).

lation, were positive in 5 of 16 dogs at 10^0 . Cultures from two of these dogs remained positive at 10^1 . Positive shell vial cultures were obtained from two of four dogs in the infection control group and three of four dogs in the group concurrently receiving an immunosuppressive dose of prednisolone. Shell vial cultures that were negative on PID 6 remained negative on PID 10.

Serologic results. *R. rickettsii*-specific antibodies were detected in all 16 dogs (Fig. 6). Although mean microimmunofluorescence antibody titers of the three treatment groups were

lower than those of the infection control group, there was no difference in mean titers among the treatment groups.

DISCUSSION

Despite frequent, concurrent use of corticosteroids and antibiotics for the treatment of acute febrile illnesses of companion animals, there are few studies that examine the potential detrimental influences of concurrent therapy when a dog has an infectious disease. We have found no studies that critically address this issue in the treatment of canine or human *R. rickettsii* infection, although cortisone has been proposed as an adjunct to chloramphenicol in the treatment of human RMSF (22). The addition of cortisone decreased the duration of fever and subjectively improved the clinical course in severely ill patients, and adverse effects were not observed (22). Based upon inference from our clinical experience, we hypothesized that immunosuppressive dosages of corticosteroids, when given concurrently with an antirickettsial antibiotic such as doxycycline, might potentiate the severity of *R. rickettsii* infection in dogs. The results of this study, which contradict this hypothesis, may have important implications for treating canine RMSF and comparative implications for the treatment of human *R. rickettsii* infection. Specifically, anti-inflammatory prednisolone therapy was not associated with any detected detrimental effects and therefore might be useful for management of some of the ocular manifestations of RMSF in dogs (4). The anti-inflammatory and immunosuppressive dosages of prednisolone administered in this study are consistent with dosages recommended for these respective indications in dogs (13). So as to enhance detection of potential prednisolone-induced detrimental effects in this study, the high end of the immunosuppressive dose range for treatment of immune-mediated thrombocytopenia was selected. Comparable immunosuppressive prednisolone dosages, generally administered intravenously for a duration of 48 to 72 h, have been recommended for initial treatment of immune-mediated thrombocytopenia in human patients (4). Based upon the results of this study, initiation of immunosuppressive prednisolone therapy in conjunction with an antirickettsial antibiotic for treatment of presumptive immune-mediated thrombocytopenia in a patient with RMSF should not induce therapeutically relevant pathophysiologic consequences.

Differentiating pathologic changes directly attributable to an infectious organism from those attributable to the host's immunologic response to the organism can be difficult. Evaluating the therapeutic utility of concurrent treatment with an

TABLE 1. Plasma disposition of doxycycline in dogs following administration of mean dose of 5.1 mg/kg every 12 h to dogs in three treatment groups^a

Trial and treatment group (n = 4)	AUC ($\mu\text{g h/ml}$)	k (h^{-1})	C_0 ($\mu\text{g/ml}$)	C_{max} ($\mu\text{g/ml}$)	Half-life ^b (h)
Trial 1					
Doxycycline	63.67 (± 4.38)	0.06 (± 0.01)	12.22 (± 0.45)	10.88 (± 0.34)	11.58 (± 1.54)
Doxycycline-anti-inflammatory prednisolone	57.00 (± 12.56)	0.12 (± 0.02)	12.80 (± 3.24)	9.85 (± 1.84)	6.22 (± 1.46)
Doxycycline-immunosuppressive prednisolone	45.92 (± 4.90)	0.13 (± 0.04)	12.19 (± 2.01)	9.18 (± 0.89)	6.68 (± 1.83)
Mean	55.53 (± 4.81)	0.10 (± 0.02)	12.40 (± 1.11)	9.97 (± 0.66)	8.16 (± 1.18)
Trial 2					
Doxycycline	113.45 (± 7.20)	0.08 (± 0.02)	23.53 (± 0.51)	17.14 (± 0.85)	9.46 (± 2.01)
Doxycycline-anti-inflammatory prednisolone	80.44 (± 15.99)	0.15 (± 0.03)	11.61 (± 0.09)	16.82 (± 3.21)	4.76 (± 0.83)
Doxycycline-immunosuppressive prednisolone	79.05 (± 15.61)	0.22 (± 0.09)	44.60 (± 31.79)	15.76 (± 3.60)	4.38 (± 1.37)
Mean	90.98 (± 8.55)	0.16 (± 0.04)	29.15 (± 13.32)	16.57 (± 1.49)	5.94 (± 1.15)

^a Treatment groups were defined by drug received: doxycycline, doxycycline with anti-inflammatory prednisolone, or doxycycline-immunosuppressive prednisolone. Doxycycline disposition was measured after initial administration (trial 1) and after 6 days of dosing (trial 2).

^b Half-life, half-life of terminal portion of plasma disposition curve.

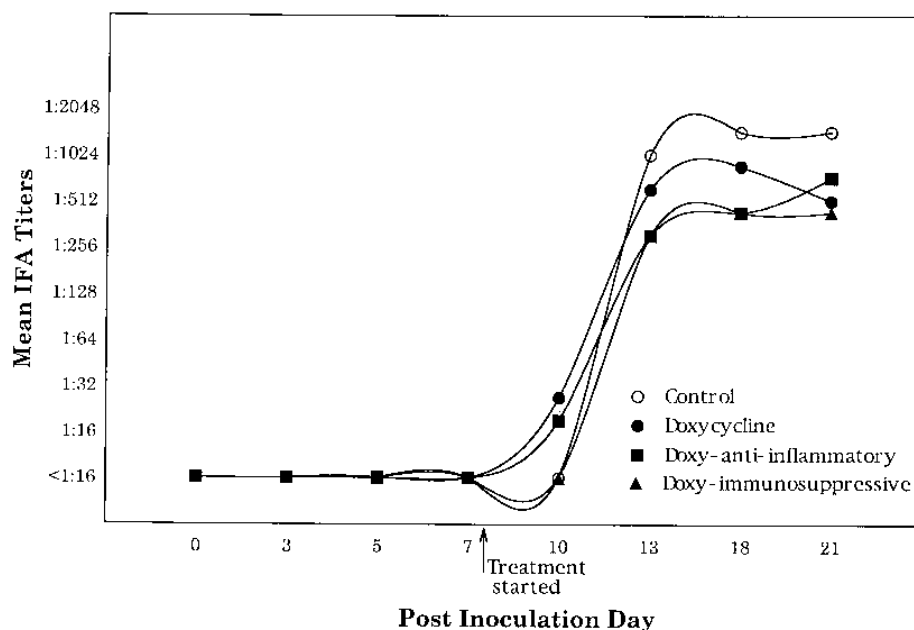


FIG. 6. Mean microimmunofluorescence antibody (IFA) titers in the control and treatment groups after inoculation with *R. rickettsii* (day 0) and after initiation of treatments (day 7).

antibiotic and corticosteroid for RMSF may be of immunopathogenic relevance because a previous study, using anti-lymphocyte serum in a guinea pig model, indicated that *R. rickettsii* causes direct cytopathic vascular injury, relatively independent of early cellular immune influences (20). Interestingly, 3 of 15 guinea pigs receiving anti-lymphocyte serum experienced an afebrile course, whereas 5 of 5 control guinea pigs developed typical terminal disease manifestations. In addition, immunosuppressed guinea pigs had microscopic vasculitis composed of predominantly neutrophils compared to the typical mononuclear lymphocytic inflammatory response in the control guinea pigs. These observations, when considered in conjunction with the results of this study, suggest that early suppression of the host's immune response to *R. rickettsii*-induced cytopathic injury might prove beneficial in the management of severe or potentially fulminant cases of RMSF. However, because of the mild to moderate severity of illness induced in these dogs, the results of this study should definitely not be construed as supporting the safety or efficacy of corticosteroids for treatment of severe canine or human RMSF.

In this study, an immunosuppressive but not anti-inflammatory dose of prednisolone prolonged the duration of rickettsemia in dogs. By PID 10, 3 days after the initiation of treatment, rickettsemia was not detected in any doxycycline or doxycycline-anti-inflammatory corticosteroid-treated dog. Similarly, in guinea pigs, anti-lymphocyte serum resulted in an increased titer of *R. rickettsii* at the terminal stage of infection, whereas rickettsiae were infrequently observed in tissues by immunofluorescent staining after 72 h of treatment with tetracycline (20). Based upon these 2 studies, it appears that both the severity (rickettsial numbers) and the duration of rickettsemia are increased by substantial suppression of the immune response. However, the slight prolongation in duration of rickettsemia (presumably days) associated with immunosuppressive prednisolone therapy was not accompanied by detrimental clinical or pathophysiologic consequences by using the test parameters described in this study. Following nonfatal experimental infection with *R. rickettsii*, dogs which are not treated

develop rapid protective immunity and are immune to rechallenge for periods up to 3 years (2). Because beagle pups treated with higher prednisolone dosages (10 mg/kg for 21 days) than used in this study developed a normal *in vivo* response to canine distemper vaccination and survived a virulent challenge (15), it seems unlikely that immunosuppressive dosages of corticosteroids would interfere with development of a protective immune response to subsequent *R. rickettsii* challenge.

For tetracyclines, the most important pharmacokinetic determinants of *in vivo* antimicrobial effects are the C_{max} and the AUC. Because these values were not different among treatments, it is not expected that the doxycycline activity in dogs varied among the treatment groups. Because doxycycline accumulated during therapy (higher C_{max} and AUC after the 10th dose than after the first dose), it is possible that doxycycline had a greater antimicrobial effect for all treatments after repeated administration than after a single administration. There was a tendency for doxycycline to be eliminated faster (i.e., shorter half-life) after the initial dose than after the 10th dose. There was a tendency for doxycycline to be eliminated more slowly (i.e., longer half-life) when it was administered alone, compared with either dose of corticosteroid, but the effect of corticosteroids on tetracycline elimination is unknown. Obviously these perceived differences did not affect the AUC or C_{max} , which are the most important determinants of therapeutic effect.

In earlier reports, the elimination rate and half-life of doxycycline have varied in dogs. Similarly, in this study, half-life varied from 1.73 to 13.4 h. Riond et al. reported a half-life of 6.99 (\pm 1.09) h and a range of 3.45 to 11.33 h after a single intravenous dose of 5 mg/kg to six dogs (17). Wilson et al. reported a half-life after a single intravenous dose of 5 mg/kg to six dogs of 10.36 h (range, 8.26 to 14.38) (21); Michel et al. reported a half-life of 10 to 12 h (14); and unpublished studies (15a) measured a half-life of 23 to 29.5 h.

Comparison of the two isolation methods used in this study was compromised due to contamination problems encountered

during the first isolation attempt. During the second isolation attempt, the shell vial centrifugation method was considerably more sensitive than isolation in flasks. However, failure to isolate rickettsiae on PID 6 from 5 of 16 dogs by the shell vial technique suggests that this modality may be accompanied by a high rate of false-negative results in the clinical setting.

R. rickettsii-specific antibody titers were lower in the treatment groups than in the infection control group. However, as in a previous study (1), doxycycline and prednisolone therapy would not interfere with serologic confirmation (seroconversion) of RMSF in dogs if appropriately timed serum samples were submitted for analysis. Similarly, cortisone administration did not appear to interfere with documentation of seroconversion in human RMSF patients (22).

Keenan and colleagues reported that the severity of disease in dogs experimentally infected with *R. rickettsii* appeared to be dose related (11). Compared to a study of similar design from our laboratory (1), the rickettsial dose in this study was increased by 1 log to facilitate development of a slightly more severe clinical course of disease so as to more critically evaluate our hypothesis. The inoculum consisted of the same *R. rickettsii* (Domino) strain, which was prepared at the same time as for the previous study and stored frozen at -70°C until utilized in this study. Despite the increase in inoculum dose, the severity of illness was not substantially increased in this study. Attitudinal scores were similar; the platelet nadir was slightly lower in this study, but there was a decrease in the mean number of retinal vascular lesions. In both studies, the dogs were laboratory-reared female beagles of similar age. Although potentially related to storage of the inoculum, reasons for the differences between the two studies are not obvious. Retrospectively, initiation of treatments on PID 5, or, as in our previous study, on PID 6, may have enhanced our ability to detect additional significant differences among the treatment groups.

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REFERENCES

- Breitschwerdt, E. B., M. G. Davidson, D. P. Aucoin, M. G. Levy, N. S. Szabados, B. C. Hegarty, A. L. Kuehne, and R. L. James. 1991. Efficacy of chloramphenicol, enrofloxacin, and tetracycline for treatment of experimental Rocky Mountain spotted fever in dogs. *Antimicrob. Agents Chemother.* **35**:2375-2381.
- Breitschwerdt, E. B., M. G. Levy, M. G. Davidson, D. H. Walker, W. Burgdorfer, B. C. Curtis, and C. A. Babineau. 1990. Kinetics of IgM and IgG responses to experimental and naturally acquired *Rickettsia rickettsii* infection in dogs. *Am. J. Vet. Res.* **51**:1312-1316.
- Breitschwerdt, E. B., D. H. Walker, M. G. Levy, W. Burgdorfer, W. T. Corbett, S. A. Hurlbert, M. E. Stebbins, B. C. Curtis, and D. A. Allen. 1988. Clinical hematologic and humoral immune response in female dogs inoculated with *Rickettsia rickettsii* and *Rickettsia montana*. *Am. J. Vet. Res.* **49**:70-76.
- Cola, C., and J. Ansell. 1990. Platelet-mediated bleeding disorders, p. 345-352. In R. E. Rakel (ed.), *Conn's current therapy*. W. B. Saunders Co., Philadelphia.
- Davidson, M. G., E. B. Breitschwerdt, M. P. Nasisse, and S. M. Roberts. 1989. Ocular manifestations of Rocky Mountain spotted fever in dogs. *J. Am. Vet. Med. Assoc.* **194**:777-781.
- Davidson, M. G., E. B. Breitschwerdt, D. H. Walker, M. G. Levy, C. S. Carlson, E. M. Hardie, C. A. Grindem, and M. P. Nasisse. 1990. Vascular permeability and coagulation during *Rickettsia rickettsii* infection in dogs. *Am. J. Vet. Res.* **51**:165-170.
- Davidson, M. G., E. B. Breitschwerdt, D. H. Walker, M. P. Nasisse, and W. E. Sussman. 1989. Identification of rickettsiae in cutaneous biopsies from dogs with experimental Rocky Mountain spotted fever. *J. Vet. Intern. Med.* **3**:8-11.
- Fishbein, D. B., M. G. Frontini, R. Giles, and L. L. Vernon. 1990. Fatal cases of Rocky Mountain spotted fever in the United States. *Ann. N.Y. Acad. Sci.* **590**:246-247.
- Grindem, C. B., W. T. Corbett, M. G. Levy, M. G. Davidson, and E. B. Breitschwerdt. 1990. Platelet aggregation in dogs experimentally infected with *Rickettsia rickettsii*. *J. Vet. Clin. Pathol.* **19**:25-28.
- Johnson, T. A., and D. W. Wichern. 1988. *Applied multivariate statistical analysis*, 2nd ed., p. 607. Prentice-Hall, Inc., Englewood Cliffs, N.J.
- Keenan, K. P., W. C. Buhles, D. L. Huxsoll, R. G. Williams, P. K. Hildebrandt, J. M. Campbell, and E. H. Stephenson. 1977. Pathogenesis of infection with *Rickettsia rickettsii* in the dog: a disease model for Rocky Mountain spotted fever. *J. Infect. Dis.* **135**:911-917.
- Marrero, M., and D. Raoult. 1989. Centrifugation-shell vial technique for rapid detection of Mediterranean spotted fever rickettsia in blood culture. *Am. J. Trop. Med. Hyg.* **40**:197-199.
- McDonald, R. K., and V. C. Langston. 1995. Use of corticosteroids and nonsteroidal anti-inflammatory agents, p. 284-293. In S. J. Ettinger and E. C. Feldman (ed.), *Textbook of veterinary internal medicine*. W. B. Saunders Co., Philadelphia, Pa.
- Michel, G., J. Mosser, and F. Fauran. 1979. Serum kinetics of doxycycline in dogs. *Eur. J. Drug Metab. Pharmacokin.* **1**:43-48.
- Nara, P. L., S. Krakowka, and T. E. Powers. 1979. Effects of prednisolone on the development of immune responses to canine distemper virus in beagle pups. *Am. J. Vet. Res.* **40**:1742-1747.
- Papich, M. G. Unpublished results.
- Riond, J.-L., and J. E. Riviere. 1990. Allometric analysis of doxycycline pharmacokinetics parameters. *J. Vet. Pharmacol. Ther.* **13**:404-407.
- Riond, J. L., S. L. Vaden, and J. E. Riviere. 1990. Comparative pharmacokinetics of doxycycline in cats and dogs. *J. Pharmacol. Ther.* **13**:415-424.
- Rowland, M., and T. N. Tozer. 1995. *Clinical pharmacokinetics: concepts and applications*, 3rd ed., p. 467-472. Appendix 1-a. Lea & Febiger, Philadelphia, Pa.
- Walker, D. H. 1990. The role of host factors in the severity of spotted fever and typhus group infections. *Ann. N. Y. Acad. Sci.* **590**:20-26.
- Walker, D. H., and F. W. Henderson. 1978. Effect of immunosuppression on *Rickettsia rickettsii* infection in guinea pigs. *Infect. Immun.* **20**:221-227.
- Wilson, R. C., D. T. Kemp, J. V. Kitzman, and D. D. Goetsch. 1988. Pharmacokinetics of doxycycline in dogs. *Can. J. Vet. Res.* **52**:12-14.
- Workman, J. B., J. A. Hightower, F. J. Borges, J. E. Furman, and R. T. Parker. 1952. Cortisone as an adjunct to chloramphenicol in the treatment of Rocky Mountain spotted fever. *N. Engl. J. Med.* **246**:962-966.