

STANDARD ARTICLE

Clinical, clinicopathologic, and gastrointestinal changes from aspirin, prednisone, or combination treatment in healthy research dogs: A double-blind randomized trial

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Abstract

Background: Dogs with immune-mediated disease are often coadministered glucocorticoids and aspirin, but ulcerogenic effects of current protocols are unknown.

Objectives: To compare gastrointestinal changes among dogs administered aspirin, prednisone, and combination treatment.

Animals: Twenty-four healthy research dogs.

Methods: Double-blinded, placebo-controlled randomized trial of dogs administered placebo, aspirin (2 mg/kg q24h), prednisone (2 mg/kg q24h), or combination treatment PO for 28 days. Clinical signs were recorded daily, with laboratory work performed at baseline and day 28. Gastrointestinal mucosal hemorrhages, erosions, and ulcers were numerated for endoscopic studies performed on days 0, 14, and 28; endoscopic mucosal lesion scores were calculated. Results were compared using mixed model repeated-measures analyses of variance and generalized estimating equation proportional odds models. $P < .05$ was considered significant.

Results: Gastric mucosal lesion scores differed by treatment-by-time ($F[6, 40] = 4.4$, $P = .002$), treatment ($F[3, 20] = 7.1$, $P = .002$), and time ($F[2, 40] = 18.9$, $P < .001$). Post hoc analysis revealed increased scores in the aspirin (day 14 only), prednisone, and prednisone/aspirin groups during treatment. Ulcers were identified on 14 studies, representing 10 dogs. Dogs receiving prednisone and prednisone/aspirin had 11.1 times (95% CI, 1.7-73.6) and 31.5 times (95% CI, 3.5-288.0) higher odds, respectively, of having endoscopic mucosal lesion scores ≥ 4 than dogs receiving placebo ($P \leq .01$).

Conclusions and Clinical Importance: Gastrointestinal bleeding occurs commonly in dogs administered aspirin, prednisone, or prednisone/aspirin treatment, with higher lesion scores for dogs receiving combination treatment. Even severe lesions are not accompanied by clinical signs.

KEYWORDS

antiplatelet, corticosteroid, gastrointestinal bleeding, glucocorticoid, thromboprophylaxis, ulcer

Abbreviations: GI, gastrointestinal; IMHA, immune-mediated hemolytic anemia; MCS, muscle condition score.

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1 | INTRODUCTION

Both sustained glucocorticoid and low-dose aspirin treatment are associated with gastrointestinal (GI) bleeding in people.¹⁻⁵ The use of other nonsteroidal medications in combination with prednisolone also causes GI bleeding in dogs.^{6,7} A study using an aspirin dose of 0.5 mg/kg q24h finds no significant difference in endoscopically identified gastric mucosal lesions between healthy dogs administered prednisone alone and prednisone with aspirin.⁸ However, the use of an aspirin dose that achieves consistent antiplatelet effects might increase GI bleeding due to additive inhibition of prostaglandin formation and potential local effects on the gastric mucosa. Although dogs often are asymptomatic in the presence of aspirin-induced GI bleeding,^{8,9} GI bleeding could increase the risk of a negative outcome, as it has been associated with increased risk of thromboembolism and death in people.¹⁰

The purpose of this randomized-controlled double-blinded study was to characterize clinical, clinicopathologic, and endoscopic changes in healthy dogs receiving sustained placebo, aspirin (2 mg/kg q24h), prednisone (2 mg/kg q24h), or combination aspirin and prednisone treatment. Our hypothesis was that sustained administration of aspirin, used singly or in combination with prednisone (2 mg/kg q24h), would induce GI bleeding, and injury would be greater for dogs that receive combination treatment than for dogs receiving prednisone alone.

2 | MATERIALS AND METHODS

2.1 | Study population

Twenty-four healthy dogs from the College's teaching and research colony were enrolled in the study. The study protocol was approved by the Institutional Animal Care and Use Committee at the University of Tennessee, College of Veterinary Medicine (protocol number 2283) and performed in compliance with "The Guide for the Care and Use of Laboratory Animals" in laboratory animal facilities that are AAALAC certified and exceed NIH standards of care.

Sample size calculation was performed using preliminary data.^{11,12} In those studies, endoscopic mucosal lesion scores¹³ increased from 5 to 15 for dogs receiving 2 mg/kg q24h prednisone with 1 mg/kg q24h aspirin. Based on those results and assuming a standard deviation of 4.9, enrollment of 6 dogs per group was calculated to have 85% power to find endoscopic scores of 5 and 15 significantly different with an alpha of .05. In contrast to prior work,¹⁴ mucosal lesion scores also increased in dogs administered prednisone alone. Dogs in the prior report were substantially younger than dogs in our pilot evaluation, potentially suggesting increasing sensitivity to the ulcerogenic effects of glucocorticoids with age. Thus, dogs were stratified by age before randomization to 1 of 4 groups using a random number sequence generator (<https://www.random.org>, accessed May 16, 2017). Because gastric biopsies taken from all dogs at the conclusion of baseline were positive for urease producing bacteria (see below), stratification based on urease status was not necessary.

2.2 | Treatment groups

The treatment groups were (1) placebo, (2) aspirin plus placebo, (3) prednisone plus placebo, and (4) prednisone plus aspirin. Dogs in the placebo group received 2 placebo capsules once daily, whereas dogs in groups 2 and 3 were administered 1 placebo capsule. Aspirin was administered at a dosage of 2 mg/kg q24h. Commercially available aspirin tablets (Rugby Laboratories, Livonia, Michigan) were compounded into capsules by the College's pharmacy using the standards for compounding provided by the United States Pharmacopeia. Prednisone was administered at a dosage of 2 mg/kg q24h using commercially available prednisone tablets (West-Ward Pharmaceuticals Corp, Eatontown, New Jersey). Lactose-containing gelatin capsules (LetCo Medical, Decatur, Alabama) were assembled by the College's pharmacy for use as placebos. All treatments were administered in small meatballs once daily before feeding by an individual blinded to the individual treatments and groups but not endoscopic findings.

2.3 | Study periods

The study was comprised of 3 periods: acclimation (days -13 to -7), baseline (days -6 to 0), and treatment (days 1-28). During the acclimation period, dogs were administered fenbendazole (50 mg/kg q24h, PO, days -13 to -9) and ivermectin (200 µg/kg SC once, day -13). As part of routine colony prophylaxis, dogs also received imidacloprid and moxidectin (Advantage Multi for dogs; Bayer HealthCare, LLC, Shawnee Mission, Kansas), dosed per manufacturer's instructions.

All dogs received water ad libitum and were fed a commercial kibble once daily in quantities sufficient to maintain ideal body condition. An observer not associated with the study and blinded to the treatment groups, medications, and all study-related findings collected observations twice daily throughout the study (days -13 to 28). Attitude was characterized as normal or abnormal. Food intake was recorded to the nearest quartile consumed (0%, 25%, 50%, or 100%). The presence of vomiting, melena, or hematochezia was recorded, and feces were scored using a standard scale.¹⁵

2.4 | Diagnostic testing

Dogs were confirmed to have negative fecal direct smears and fecal flotations (sugar and zinc sulfate) during the acclimation period (days -13 to -11). Clinicopathologic testing was performed at the conclusion of baseline and treatment by a commercial diagnostic laboratory (Antech Diagnostics, Fountain Valley, California). Testing included CBCs, serum biochemical profiles with lipase activity (PrecisionPSL; Antech Diagnostics), urinalyses, and urine protein:creatinine ratios.

Dogs were anesthetized on days 0, 14, and 28 for performance of esophagogastroduodenoscopy. Dogs were premedicated with acepromazine (.02 mg/kg SC) and butorphanol (.4 mg/kg SC) after which IV catheters were placed, and dogs were induced using propofol (3-6 mg/kg IV to effect). Dogs were intubated, and general anesthesia was maintained with isoflurane administered in oxygen. Crystalloid

fluids were administered at a rate of 10 mL/kg/h IV. Dogs then were positioned in left lateral recumbency.

Esophagogastroduodenoscopy was performed by a single individual (J.C.W.), blinded to each dog's treatment group, using a standardized technique to avoid creation of iatrogenic lesions.^{8,13} Briefly, endoscopic exploration was performed under continuous visual guidance from the upper esophageal sphincter through the duodenum. Within the stomach, the gastric body, antrum/pylorus, angularis incisura, and cardia were individually interrogated before pyloric intubation for duodenal evaluation. Endoscopic explorations were recorded using a digital capture system, with still images collected of the lower esophageal sphincter, gastric body, angularis incisura, antrum and pylorus, cardia, and duodenum. Supplementary images of focal abnormalities also were collected. Endoscopic evaluations were anonymized after data collection to blind investigators to dog, treatment group, and time point. Gastric biopsies taken on day 0 were incubated in urease media to assess for the presence of potential *Helicobacter* spp.

2.5 | Endoscopic scoring

Two investigators (J.T., J.C.W.) independently evaluated each endoscopic study. Hemorrhages, punctate erosions, invasive erosions, and ulcers were numerated for the esophagus, each region of the stomach (gastric body, pyloric antrum, angularis incisura, and cardia), and duodenum.^{6,8} Hemorrhages were defined as reddened areas with intact mucosa. Pinhead-sized or smaller discontinuations in the mucosa were classified as punctate erosions, whereas discontinuations greater than a pinhead in size or with detectable depth were classified as invasive erosions. Lesions with wide mucosal defects and craterous centers were classified as ulcers. If >25 lesions were identified in an anatomic region, lesions were recorded as 26-50, 51-100, 100-200, or >200 to avoid erroneous quantitation. For statistical analysis, lesions >25 but ≤200 were entered according to the midpoint of the categorical range (eg, 26-50 was entered as 37.5, and so on). When >200 lesions were identified, a value of 201 was assigned.

To optimize consistency in endoscopic evaluation given high reported interindividual variation in endoscopic interpretation,¹⁶ investigators independently evaluated 10 videos and still images of 10 GI endoscopies unrelated to this study 4 months after completion of data collection. Studies were selected to represent a range of GI disease, from normal to severe GI bleeding, by 1 investigator (J.C.W.) before the start of the study. After datasheets from the individual investigators were merged, the combined database was reviewed for areas of discordance. Each investigator then independently reevaluated studies with discordant scores and corrected any self-identified errors in scoring or data entry. Finally, the 2 investigators reviewed the studies together to address areas of disagreement and reach consensus in differentiation among lesion types.

Anonymized endoscopic evaluations for this study were independently scored thereafter (approximately 6 months after completion of data collection). After datasheets from the individual investigators were merged, the combined database was reviewed for areas of discordance. Each investigator then independently reevaluated studies

with discordant scores and corrected any self-identified errors in scoring or data entry. Results of these studies were not reviewed together to achieve consensus. Instead, the mean of lesions numerated by each of the 2 investigators was used for analyses. Total gastric mucosal endoscopic lesion scores were calculated based on mean lesion counts using the Forsyth scoring system.⁶

2.6 | Statistical and data analysis

Descriptive statistics were generated for relevant clinical, clinicopathologic, and endoscopic variables. Samples were analyzed for normality using the Shapiro-Wilk test and for the presence of outliers using box-and-whisker plots.

Mean food intake, days of vomiting, and mean fecal score were determined for each study period. Clinicopathologic variables recorded were hematocrit and platelet count; albumin and blood urea nitrogen (BUN) concentrations; BUN:creatinine and urine protein:creatinine ratios; activities of alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), amylase, and lipase; and urine-specific gravity.

Selected clinical, clinicopathologic, and endoscopic data were compared between treatment groups using mixed model, split-plot repeated-measures analyses of variance that included fixed effects of treatment, time, and treatment-by-time interaction. The repeated measure of time was accounted for in a repeated statement. Dog nested within treatment group was included as a random effect. Fisher's least significant difference was used to perform post hoc analyses. The Shapiro-Wilk test of normality and QQ plots of the residuals were evaluated for each marker to confirm the assumption of normally distributed residuals had been met. Model assumptions regarding equality of variances were verified with Levene's Test for equality of variances. Differences in marginal means were determined for markers with significant main effects or interaction terms. Non-normally distributed data were logarithmically or rank-transformed, as necessary, to meet underlying statistical assumptions. If logarithmic transformation was required, 0.05 was added to all values. Due its infrequent occurrence, mixed model analysis could not be performed for ulceration. The relative odds of having a total endoscopic mucosal lesion score ≥4 (eg, >25 hemorrhages or punctate erosions, ≥1 invasive erosion, and/or ≥1 ulcer) was determined using a repeated-measures generalized estimating equation proportional odds model with a binomial distribution and a logit link function. After data analysis revealed a lack of association between hemorrhages and group or time point (see Results), hemorrhages were excluded from lesion scores in order to increase precision of the results.

Commercial statistical software packages (MedCalc 15.8 MedCalc Software, Ostend, Belgium; SAS 9.4 release TS1M5, SAS Institute Inc, Cary, North Carolina) were used for all analyses. $P < .05$ was considered significant.

TABLE 1 Baseline demographics of dogs stratified by age then randomized to receive placebo, aspirin with placebo, prednisone with placebo, or a combination of prednisone and aspirin for 28 days

	Placebo	Aspirin	Prednisone	Prednisone and aspirin
Age (y)	3.5 (2-6)	3.5 (2-7)	3.0 (2-6)	3.5 (2-7)
Sex				
Intact female	3	2	2	2
Female spayed	0	0	0	0
Intact male	1	2	2	3
Male castrated	2	2	2	1
Breed	3 Beagles, 3 Hounds	4 Beagles, 2 Hounds	4 Beagles, 2 Hounds	4 Beagles, 2 Hounds
Weight (kg)	14.2 ± 3.1	15.8 ± 7.1	15.2 ± 6.1	20.9 ± 9.9
Body condition score	6 (5-8)	6 (4-8)	6 (5-9)	7 (5-8)
Muscle condition score	3 (NA)	3 (2-3)	3 (NA)	3 (NA)

Age, body condition score, and muscle condition score are presented as median (range). Weight is presented as mean ± SD.

Abbreviation: NA, not applicable.

3 | RESULTS

3.1 | Study population

Baseline demographics of the 4 treatment groups are summarized in Table 1. Attitude was categorized as normal on all days for all dogs. There was no significant difference in food intake or body condition score among groups over time. All but 3 dogs had a muscle condition score (MCS) of 3 at all time points. One dog in the aspirin group had an MCS of 2 at all time points. Two dogs (aspirin, 1; prednisone, 1) had MCSs of 3 at baseline but 2 on day 28. Six dogs vomited at least once during the study, representing 2 dogs each in the placebo, aspirin, and prednisone/aspirin groups. No dog in the prednisone group experienced vomiting. Vomiting was noted once during baseline for 3 dogs (1, placebo; 2, prednisone/aspirin). During the treatment period, vomiting was recorded on 1 day for 3 dogs (1, aspirin; 2, prednisone/aspirin), on 2 days for 1 dog (aspirin), and on 3 days for 1 dog (placebo). Neither hematemesis or melena nor hematochezia occurred during the study. No dog developed diarrhea, and fecal scores did not differ among groups over time.

3.2 | Clinicopathologic data

Selected baseline and post-treatment clinicopathologic results are presented in Table 2. One dog in the prednisone group had a mildly low platelet count (143,000 platelets/ μ L) at baseline, but platelet clumping was noted. Both manual review and a repeat platelet count the next day yielded platelet counts within the reference interval. Results of CBCs otherwise were unremarkable for all dogs. Results for biochemical analytes also were within reference intervals at both time points for the majority of dogs. Logarithmic transformation was required before statistical analysis for ALP and lipase activity, creatinine concentration, and urine protein:creatinine ratio, whereas rank transformation was required before analysis of GGT activity and BUN concentration.

Serum ALP activities increased over time in the prednisone and prednisone/aspirin groups, resulting in a significant treatment-by-time interaction ($F[3, 20] = 10.8, P < .001$) and significant corresponding post-hoc tests ($P < .001$ for each). Both GGT ($F[1, 20] = 10.5, P = .004$) and lipase ($F[1, 20] = 10.3, P = .004$) activities increased significantly over time due to increased activities in the prednisone-receiving groups although the treatment-by-time interactions were not significant. Amylase activities decreased over time for the prednisone and prednisone/aspirin groups, resulting in a treatment-by-time interaction ($F[3, 20] = 5.8, P = .006$) and significant corresponding post hoc tests ($P \leq .002$ for each).

Although neither BUN concentration nor urine-specific gravity differed among treatments or over time, BUN:creatinine ratios significantly differed by treatment-by-time ($F[3, 20] = 5.6, P = .006$) and time ($F[3, 20] = 5.8, P < .001$). Post hoc tests revealed significant increases in BUN:creatinine ratios over time for prednisone and prednisone/aspirin treatment groups compared to baseline values ($P < .001$ for each). Significant changes over time were not observed in BUN:creatinine ratios for dogs treated with aspirin or placebo. Differences in BUN:creatinine ratios over time among groups reflected significant decreases in creatinine concentrations ($F[3, 20] = 6.48, P = .003$). Post hoc tests revealed that creatinine concentrations in dogs treated with prednisone or prednisone/aspirin significantly decreased over time ($P \leq .001$ for each), while similar decreases were not observed for dogs treated with aspirin or placebo. Finally, urine protein:creatinine ratios were significantly higher after treatment for the prednisone and prednisone/aspirin groups compared to baseline values and results for the other 2 groups, as demonstrated by both significant treatment-by-time interactions ($F[3, 20] = 5.9, P = .005$) and corresponding post hoc tests ($P \leq .01$ for each).

3.3 | Endoscopic findings

Gastric biopsies taken at baseline from all dogs were positive for urease-producing bacteria.

TABLE 2 Selected clinicopathologic results for 24 healthy research dogs administered placebo, aspirin with placebo, prednisone with placebo, or a combination of prednisone and aspirin for 28 days

	RI	Placebo		Aspirin		Prednisone		Prednisone and aspirin	
		Baseline	Day 28	Baseline	Day 28	Baseline	Day 28	Baseline	Day 28
Amylase (IU/L)	290-1125	435 ^{ABC} (316-762)	544 ^A (363-852)	476 ^{AB} (355-854)	391 ^{ABC} (326-708)	409 ^{AB} (310-950)	245 ^C (225-509)	524 ^A (395-848)	354 ^{BC} (264-458)
Albumin (g/dL)	2.7-4.4	3.8 ^{ABC} (3.3-4.2)	3.7 ^{ABC} (3.5-4.1)	3.8 ^{ABC} (3.1-3.9)	3.6 ^{ABC} (3.4-3.7)	3.5 ^{BD} (3.3-3.9)	4 ^{AC} (3.3-4.2)	3.6 ^{CD} (3.2-3.9)	4.1 ^{AB} (3.4-4.1)
ALP (IU/L)	5-131	24 ^B (18-34)	26 ^B (19-42)	32 ^B (20-34)	40 ^B (23-67)	32 ^B (26-91)	103 ^A (48-161)	25 ^B (15-47)	124 ^A (42-509)
GGT (IU/L)	1-12	5* (4-8)	7* (5-7)	6* (4-7)	5* (1-7)	5* (5-7)	8* (6-10)	5* (3-12)	9* (4-24)
BUN (mg/dL)	6-31	12 (8-36)	14.5 (9-20)	12 (10-21)	13.5 (11-23)	18 (10-20)	17.5 (13-22)	13 (11-14)	15 (11-19)
Creatinine (mg/dL)	0.5-1.6	0.8 ^{ABC} (0.7-1.4)	0.7 ^{CDE} (0.6-0.9)	0.8 ^{ADC} (0.7-1.1)	0.8 ^{BCD} (0.6-1.1)	1 ^A (0.8-1.4)	0.7 ^{DE} (0.6-0.7)	0.9 ^{AB} (0.6-1.5)	0.6 ^E (0.5-0.7)
BUN:creatinine ratio	4-27	16 ^{BC} (11-26)	20.5 ^B (13-27)	16 ^{BC} (13-19)	18 ^{BC} (14-28)	15.5 ^A (13-19)	27 ^{BC} (22-31)	15 ^C (7-20)	27 ^A (22-28)
Hematocrit (%)	36-60	55* (46-63)	53* (48-62)	54* (48-59)	53* (44-59)	54* (48-57)	49* (43-54)	48* (42-64)	44* (37-56)
Lipase (U/L)	24-140	40.5* (16-100)	51.5* (18-346)	42* (20-88)	40.5* (27-170)	45.5* (29-120)	95.5* (38-177)	35.5* (16-114)	114* (21-254)
Platelet count (x10 ³ /μL)	170-400	291 (192-417)	240 (183-339)	304 (194-348)	262 (218-419)	238 (143-317)	256 (173-421)	275 (199-414)	292 (200-461)
Urine-specific gravity	1.015-1.050	1.023 (1.004-1.050)	1.026 (1.014-1.041)	1.029 (1.011-1.041)	1.035 (1.015-1.050)	1.028 (1.005-1.038)	1.019 (1.002-1.026)	1.029 (1.012-1.042)	1.020 (1.004-1.028)
Urine protein:creatinine ratio	<0.5	0.1 ^C (0.1-0.4)	0.2 ^C (0.1-0.5)	0.3 ^{BC} (0.1-0.6)	0.2 ^C (0.1-0.4)	0.3 ^C (0.1-1.5)	0.6 ^{AB} (0.2-4.1)	0.3 ^{BC} (0.1-0.6)	1.7 ^A (0.1-2.8)

Results are presented as median (range). Superscript letters and asterisks highlight values with significant treatment-by-time, treatment, or time interactions. Values that do not share a common superscript letter differed significantly ($P < .05$) based on post hoc analysis. * indicates values that differed significantly ($P < .05$) over time independent of treatment.

Abbreviations: ALP, alkaline phosphatase; BUN, blood urea nitrogen; GGT, gamma-glutamyl transferase; RI, reference interval.

Median (range) numbers of gastric mucosal lesions are summarized in Table 3. Hemorrhages, punctate erosions, and invasive erosions required logarithmic transformation before statistical analysis in order to meet underlying statistical assumptions. Total mucosal hemorrhages did not differ among treatment groups or over time ($P > .2$). Total gastric punctate erosions had significant treatment-by-time ($F[6, 40] = 3.0, P = .02$), treatment ($F[3, 20] = 10.0, P < .001$), and time ($F[2, 40] = 14.4, P < .001$) effects. Additionally, total gastric invasive erosions had significant treatment-by-time ($F[6, 40] = 5.8, P < .001$), treatment ($F[3, 20] = 11.7, P < .001$), and time ($F[2, 40] = 27.2, P < .001$) effects. Post hoc analysis

TABLE 3 Median (range) for gastric mucosal lesions identified on endoscopy for 24 healthy research dogs administered placebo, aspirin with placebo, prednisone with placebo, or a combination of prednisone and aspirin for 28 days

	Baseline	Day 14	Day 28
Placebo			
Hemorrhages	0 (0-29)	3 (0-132)	1 (0-10)
Punctate erosions	0 (0-14) ^D	0 (0-1) ^D	0 (0-36) ^{CD}
Invasive erosions	0 (0-4) ^E	0 (0-1) ^E	0 (0-11) ^E
Ulcers ^a	0 (0-0)	0 (0-0)	0 (0-1)
Aspirin			
Hemorrhages	0 (0-60)	16 (0-31)	0 (0-16)
Punctate erosions	0 (0-7) ^{CD}	14 (0-37) ^{BC}	0 (0-227) ^{CD}
Invasive erosions	0 (0-1) ^E	3 (0-36) ^{CD}	0 (0-41) ^{DE}
Ulcers ^a	0 (0-0)	0 (0-22)	0 (0-4)
Prednisone			
Hemorrhages	0 (0-17)	1 (0-10)	7 (0-105)
Punctate erosions	0 (0-2) ^D	37 (0-303) ^{AB}	55 (7-261) ^{AB}
Invasive erosions	0 (0-0) ^E	10 (0-45) ^{BC}	33 (3-211) ^{AB}
Ulcers ^a	0 (0-0)	0 (0-1)	0 (0-1)
Prednisone and aspirin			
Hemorrhages	5 (0-64)	0 (0-2)	0 (0-5)
Punctate Erosions	0 (0-237) ^{CD}	214 (10-540) ^A	164 (1-220) ^A
Invasive Erosions	0 (0-16) ^E	85 (16-220) ^A	13 (5-100) ^{AB}
Ulcers ^a	0 (0-0)	3 (0-25)	1 (0-3)

Values that do not share a common superscript letter differed significantly ($P < .05$) among groups over time based on post hoc analysis.

^aStatistical comparisons were not performed due to limited occurrences.

revealed that differences were due to greater numbers of lesions during treatment for dogs in the aspirin (invasive erosions, day 14 only), prednisone (days 14 and 28), and prednisone/aspirin groups (days 14 and 28; $P \leq .008$ for all comparisons). Erosion often was marked and multifocal (Figure 1), although primarily concentrated in the antrum. Ulceration was noted in 14 studies, representing 10 dogs over the course of the study (Table 3, Figure 2). Ulcers were generally limited in size and depth, but severe ulceration was noted in 2 dogs (Video S1). One dog in the placebo group had 1 small ulcer identified on day 28. One dog in the aspirin group had 22 ulcers identified at day 14. The same dog had 4 ulcers at day 28. It was not possible to determine whether these were persistent or new ulcers due to the severity of ulceration on day 14. Three dogs in the prednisone group each developed 1 ulcer (day 14, 1 dog; day 28, 2 dogs). Finally, 5 dogs in the prednisone/aspirin group had between 1 and 25 ulcers identified at day 14; 2 of the same dogs had 1 and 3 new ulcers identified on day 28, respectively. Residual gastric contents were noted in the majority of dogs with GI bleeding. Duodenal lesions were rare, limited to hemorrhages (maximum score, 3) and invasive erosions (maximum score, 2) in 5 dogs each.

As shown in Figure 3, total gastric endoscopic mucosal lesion scores¹³ differed significantly by treatment-by-time ($F[6, 40] = 4.4, P = .002$), treatment ($F[3, 20] = 7.1, P = .002$), and time ($F[2, 40] = 18.9, P < .001$). Post hoc analysis revealed significantly higher lesion scores during treatment over time for dogs in the aspirin (day 14 only), prednisone (days 14 and 28), and prednisone/aspirin groups (days 14 and 28) ($P \leq .02$ for all comparisons). Scores for dogs in the prednisone/aspirin group on day 14 were significantly higher than those for any other time point or in any other group ($P \leq .05$).

Forty studies had endoscopic mucosal lesion scores ≥ 4 , with significant differences among treatment groups ($\chi^2 [3] = 18.05, P < .001$). Dogs receiving prednisone and prednisone/aspirin had 5.1 times (95% CI, 1.4-18.1) and 11.2 times (95% CI, 3.4-37.0) higher odds of developing total lesion scores ≥ 4 over time than dogs administered placebo ($P \leq .01$), respectively. When hemorrhages were excluded from scoring of endoscopic mucosal lesions, odds of total lesions scores ≥ 4 occurring were 11.1 times (95% CI, 1.7-73.6) and 31.5 times (95% CI, 3.5-288.0) times higher for dogs receiving prednisone or prednisone/aspirin, respectively, than placebo ($P \leq .01$). In contrast, dogs receiving aspirin were not more likely to have lesion scores ≥ 4 over time than dogs administered placebo.

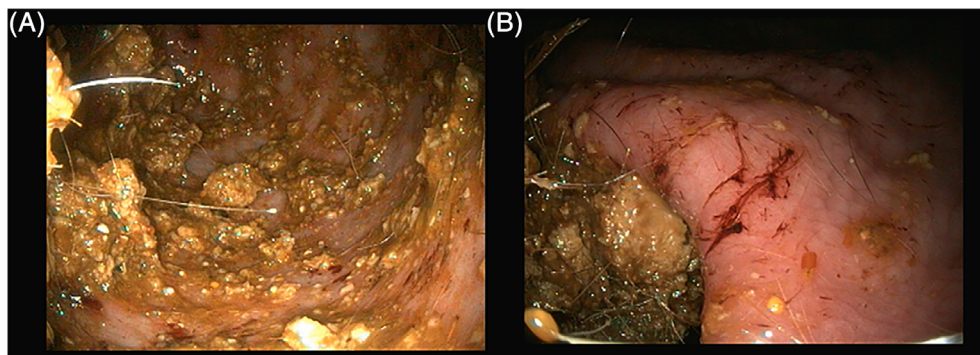


FIGURE 1 Diffuse punctate and invasive erosions in the antrum (A) and angularis incisura (B) of a healthy dog administered prednisone (2 mg/kg q24h) and aspirin (2 mg/kg q24h) PO for 14 days. Residual gastric contents in the antrum (A) likely resulted in underestimation of number and depth of some lesions

FIGURE 2 Healing (black arrow) and active ulceration with adherent clots (white arrows) in the gastric body of healthy dogs administered (A) prednisone (2 mg/kg q24h) and aspirin (2 mg/kg q24h) PO for 28 days and (B) aspirin (2 mg/kg q24h) PO for 14 days

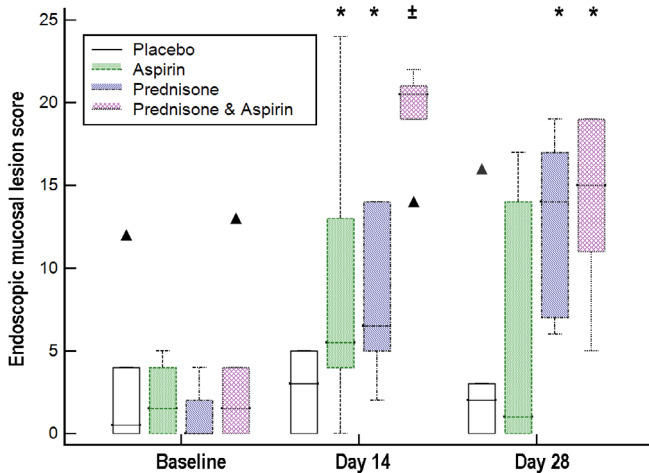
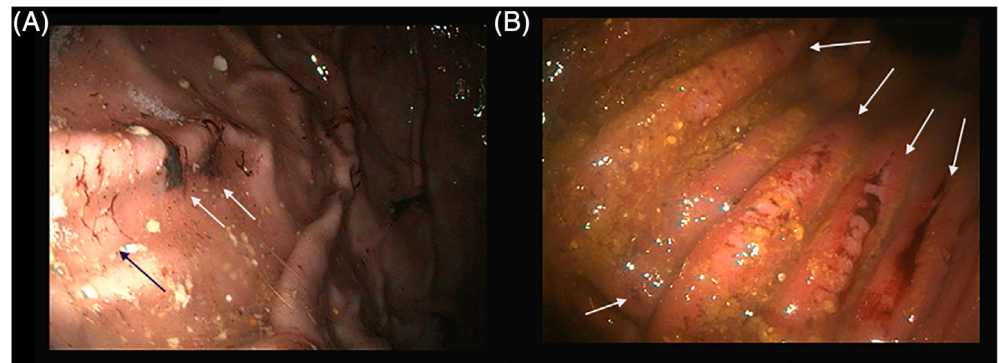


FIGURE 3 Box and whisker plots of total gastric endoscopic mucosal lesion scores⁶ for 24 healthy dogs randomized to receive placebo, aspirin with placebo, prednisone with placebo, or a combination of prednisone and aspirin for 28 days. ▲ = outliers, * = significantly higher than scores without overlying symbols, † = significantly higher than all other scores

4 | DISCUSSION

Total gastric endoscopic mucosal lesion scores increased significantly in dogs administered aspirin (day 14 only), prednisone, and prednisone/aspirin combination treatment in this study. Increases were primarily due to increased numbers of punctate and invasive erosions, although ulcers developed in 10 dogs over the course of the study. Both numbers of lesions and endoscopic lesion scores were higher on day 14 than 28, with significantly higher values in the aspirin and prednisone/aspirin group on day 14 compared to day 28. However, a number of dogs had new lesions, such as ulcers, identified on day 28, suggesting ongoing primary disease. Dogs receiving prednisone and prednisone/aspirin combination treatment had 11.1 times and 31.5 times higher odds to have endoscopic lesions scores ≥ 4 (eg, >25 punctate erosions, ≥ 1 invasive erosion, ≥ 1 ulcer, or all) than dogs administered placebo.

Although gastric mucosal damage was marked and often severe, it was not accompanied by changes in food intake, vomiting, fecal score, or weight. Furthermore, many clinicopathologic changes associated with GI bleeding were not identified, potentially due to offsetting effects of glucocorticoids. For example, permissive effects of steroids on

erythropoiesis could have blunted development of anemia secondary to GI blood loss. Lack of hypoalbuminemia could reflect increased albumin synthesis secondary to steroid administration, as occurs in both experimental models and people with hepatic and non-hepatic disease.^{14,17-21} Other glucocorticoid-associated changes identified in this study included decreased amylase activity; increased ALP, GGT, and lipase activity; and increased urine protein:creatinine ratio compared to baseline. Though results for these analytes changed significantly over time and differed from those for other groups, results uncommonly were out of the reference interval in spite of an extended duration of glucocorticoid administration. The exception was urine protein:creatinine ratios, for which median values were above the reference interval at day 28 for dogs in the prednisone and prednisone/aspirin groups.

The BUN:creatinine ratio detects changes consistent with GI bleeding in absence of increases in BUN concentration, given the latter's poor sensitivity for bleeding. Ten percent of people experiencing acute GI blood loss of ≥ 1.1 L have BUN concentrations <24 mg/dL.²² Furthermore, only 66% of subjects with massive GI hemorrhage have BUN concentrations ≥ 31 mg/dL—the upper limit of the reference interval for BUN in this study. Less marked increases are anticipated in cases with chronic compensated blood loss,²³ like that identified in our study. The median BUN:creatinine ratio for dogs with overt upper GI bleeding is 28,²³ with no difference in BUN:creatinine ratios between dogs that have received steroids versus those that have not. Although BUN concentrations did not change in this study, median BUN:creatinine ratios increased by 1.8-fold to 27 in the prednisone-containing groups—almost identical to median ratios for dogs in the prior report. Given the lack of changes in MCS in spite of decreased creatinine concentrations in the prednisone-receiving groups, it is reasonable to surmise that glucocorticoid-induced polydipsia offset GI bleeding-associated increases in BUN concentrations. Unfortunately, this hypothesis cannot be confirmed because neither urine output nor water intake was quantitated. Although urine-specific gravity did not differ significantly among groups, first morning voided urine samples could not be collected because the majority of the dogs urinated at will in their runs.

Glucocorticoids remain the mainstay of treatment for IMHA²⁴ due to their rapid onset of action, proven efficacy, and low cost. Glucocorticoids have complex and competing effects on the GI tract. Basal concentrations help maintain GI mucosal integrity, and stress-related increases in endogenous glucocorticoid concentrations are gastroprotective due to

maintenance of local glucose homeostasis.^{1,2} Sustained glucocorticoid excess, however, inhibits endogenous peroxidase,²⁵ which scavenges endogenous hydrogen peroxide in the gastric mucosa.²⁶ Peroxidase is rich in the parietal cell and also plays a role in the control of acid secretion.²⁷ Inactivation of peroxidase leads to increased free radical damage, decreased synthesis of prostaglandin, hyperacidity, and altered vascular permeability, ultimately resulting in GI ulceration.^{1,2,26-33}

Short-term injectable corticosteroid administration can cause GI bleeding and ulceration in healthy dogs.³⁴⁻³⁶ Almost all (90%-100%) dogs administered high-dose methylprednisolone IV for 48 hours in 2 studies develop severe GI bleeding, with disease primarily located in the antrum.^{34,35} The first³⁴ of these studies finds GI bleeding associated with residual gastric contents, consistent with results of our study. Lack of an association between bleeding and gastric contents in the second study likely reflects an increased fasting period before anesthesia.³⁵ Healthy dogs administered high-dose twice daily dexamethasone SC for 8 days also develop severe GI bleeding with lesions primarily located in the antrum and minimal difference in the severity of lesions between days 2 and 8.³⁶ Additionally, dogs receiving dexamethasone develop anemia and loose dark feces, neither of which is reported in dogs administered methylprednisolone. Fecal occult blood tests are positive in the majority of dogs with GI bleeding.³⁴⁻³⁶ Finally, GI bleeding increases significantly for middle-aged healthy research dogs administered high-dose prednisolone for 3 days, with vomiting at least once during treatment occurring in 83% of dogs.³⁷ In contrast to these 4 studies, sustained oral administration of prednisone is not associated with an increase in the incidence or severity of GI bleeding in healthy dogs in another randomized trial.⁸

Potential explanations for conflicting findings among the prior studies, as well as ours, include differences in the drugs administered, dosages used, routes of administration, and durations of treatment. However, none of these possibilities explains the discordance between the results of our study and another study using the same corticosteroid, dosage, and duration of treatment.⁸ Furthermore, all dogs in both studies had positive test results for *Helicobacter* spp. Commercial prednisone tablets were used for this study versus being compounded into gelatin capsules.⁸ In this study, aspirin similarly was repackaged into capsules due to its instability in liquid formulations³⁸ versus being suspended in almond oil.⁶ Discordance in findings regarding the impact of aspirin administration on GI bleeding, thus, could reflect differences in aspirin dose, differences in drug reformulation, or both. It is possible, though exceedingly unlikely, that differences in drug formulation contributed to the discordant association between prednisone administration and GI bleeding between the 2 studies. A more likely explanation is that the discordance between studies reflects differences in subject age. Although 3 prior studies do not provide subject age,³⁴⁻³⁶ median age of the dogs administered prednisone without associated GI bleeding is substantially younger (14 months)⁸ than that of the dogs in our pilot evaluation (48 months) or the study that provides age (58 months),³⁷ potentially suggesting an increasing sensitivity to the ulcerogenic effects of glucocorticoids with age. To prevent age from potentially confounding our results, dogs with a wide range of ages were used, and subjects were stratified by age before group randomization. Covariate

testing revealed a lack of association between age and development of bleeding, but the lack of an age-related association must be interpreted with care given small group sizes.

Survival rates in dogs with immune-mediated hemolytic anemia (IMHA) historically are low (30%-50%),³⁹ primarily due to fatal thromboembolism. As such, current recommendations for IMHA include the immediate initiation of thromboprophylactic treatment.^{24,40} Thromboprophylaxis should continue until the resolution of the hypercoagulable state because premature cessation of thromboprophylaxis is associated with an increased risk of thrombosis.²⁴ Because glucocorticoids independently increase thrombotic risk,⁴⁰⁻⁴² ACVIM guidelines for dogs with IMHA recommend thromboprophylaxis continue until dogs are weaned off prednisone.²⁴ Given the need for prolonged treatment and in the absence of objective data showing superiority of 1 thromboprophylactic agent for management of canine IMHA, drug selection often is influenced by financial and lifestyle constraints of owners. Given its low cost, oral route of administration, once daily dosing interval, and long-term efficacy in decreasing mortality in 1 retrospective report of dogs with IMHA,³⁹ aspirin continues to be widely prescribed for IMHA. The ideal dose, however, remains unclear. The dosage of aspirin shown to decrease mortality in the aforementioned retrospective study does not result in reliable platelet inhibition based on platelet function tests.^{43,44} Reliable inhibition requires an aspirin dosage of 2 mg/kg q24h.⁴⁴

Aspirin exerts its antiplatelet effects through irreversible acetylation of the COX active site in platelets and megakaryocytes, decreasing thromboxane production and impairing platelet response.³ Due to non-selective COX inhibition, aspirin administration can cause gastric ulceration and intestinal lesions in dogs in a dose-dependent manner.^{6,9,45} Aspirin also increases intestinal permeability and decreases recovery of barrier function after ischemic insult.⁴⁶ These effects are particularly concerning in dogs with IMHA, because of the local ischemic effects of marked anemia. Occult GI bleeding is the major negative side effect of low-dose aspirin treatment in people.³⁻⁵ Systematic reviews find no association between the type of aspirin formulation (plain, enterically coated, buffered) and relative risk of GI bleeding in people.⁴⁷ As such, minimizing the aspirin dose is the best way to decrease the risk of bleeding. Although 1 prior report finds no association between administering low-dose aspirin with prednisone and GI bleeding in healthy dogs,⁸ the aspirin dosage used in that study is much lower (.5 mg/kg q24h) than the currently recommended dosage for antiplatelet effects (2 mg/kg q24h).⁴⁴ In this study, aspirin administration significantly increased gastric mucosal lesions on day 14, with development of profound and sustained ulceration in 1 dog (Figure 4, Video S1). Furthermore, the administration of combination of prednisone/aspirin treatment more than doubled the risk of having endoscopic mucosal lesion scores ≥ 4 compared to that for dogs administered prednisone alone.

Unfortunately, neither misoprostol, omeprazole, or sucralfate nor cimetidine administration decreases the incidence or severity of GI bleeding due to short-term glucocorticoid treatment in healthy dogs or dogs with intervertebral disk disease.^{35,48,49} Omeprazole is ineffective in decreasing GI bleeding in dogs due to sustained glucocorticoid

and low-dose aspirin treatment based on 1 preliminary report,¹¹ although the omeprazole dosage used was less than that currently recommended for treatment of GI ulceration.⁵⁰ Thus, prophylactic administration of these gastroprotectants is not recommended given their lack of efficacy.⁵⁰ In contrast, mosapride decreases both GI bleeding and the occurrence of vomiting due to short-term administration of high-dose prednisolone.³⁷ Further evaluation is warranted to determine whether similar prophylactic benefits occur in dogs administered sustained oral glucocorticoid treatment. In cases where GI bleeding is confirmed, management using twice daily proton pump inhibitor treatment is warranted to improve healing.⁵⁰ Based on the results of this study, however, the primary focus should be on reducing or eliminating glucocorticoid treatment.

Results of this study cannot be extrapolated to the use of prednisone with other thromboprophylactic agents. Although clopidogrel, heparin, and direct oral anticoagulants increase the risk of GI bleeding in people,^{4,51-54} the underlying etiopathology for each differs from that of prednisone-induced bleeding, aspirin-induced bleeding, or both. Furthermore, results for people might not accurately predict their effects in dogs. Fecal occult blood testing was not performed in this study, which might have enhanced detection of occult GI bleeding. However, false positives occur due to differences in diet,⁵⁵ potentially limiting application of test results in client-owned animals. Other limitations of our study include the use of healthy research dogs and the relatively short study duration. Although dogs ranged in age and were older than dogs in 1 prior report, they all were either Beagles or Hound dogs, and none had underlying disease. Results might differ in dogs with underlying disease or receiving additional medications. Subtle changes in attitude, appetite, and fecal quality might have been overlooked given the lack of 24-hour surveillance and owner-pet pair bonding. Although GI lesion scores were equivalent to or higher for dogs administered prednisone/aspirin combination treatment versus prednisone alone, hematocrit did not differ significantly between those groups. In fact, anemia was not noted in any dog, despite the occurrence of multifocal punctate erosions, invasive erosions, and ulceration (Table 3). The short study duration could have contributed to a lack of difference in hematocrit, because anemia due to chronic GI bleeding can take months to manifest in an animal with normal iron stores. Gastrointestinal bleeding significantly decreased between days 14 and 28 for dogs receiving aspirin and prednisone/aspirin combination treatment. It is possible that prednisone-associated GI bleeding could have resolved with ongoing administration, although this seems unlikely given the development of new lesions, including ulcers, by day 28 in several dogs. Finally, the impact of these findings on managing dogs with immune-mediated diseases is unknown. Further evaluation in dogs receiving sustained treatment for naturally occurring diseases will be necessary to determine the impact of GI bleeding on disease management, thromboembolic risk, and long-term survival.

In conclusion, oral administration of prednisone and prednisone/aspirin combination treatment increased the risk of having endoscopic lesions scores ≥ 4 (eg, >25 punctate erosions, ≥ 1 invasive erosion, ≥ 1 ulcer, or all) 11.1- and 31.5-fold, respectively, in this study. Lesions were most severe on day 14 for dogs receiving prednisone/aspirin

combination treatment, with no significant difference in the amount or severity of lesions on day 28 between dogs receiving prednisone versus combination treatment. Even when severe, GI bleeding was not accompanied by changes in attitude, food intake, vomiting, fecal score, hematocrit, or BUN concentration in dogs. Neither hematemesis nor melena was noted. Clinicians must maintain a heightened suspicion for GI bleeding; changes in the BUN:creatinine ratio might be helpful in identifying dogs experiencing occult bleeding without classical biochemical changes, such as increased BUN concentration or anemia. When possible, glucocorticoids should be weaned before other immunomodulators to minimize occult GI blood loss and facilitate discontinuation of thromboprophylactic treatment.

CONFLICT OF INTEREST DECLARATION

The funder, University of Tennessee, Knoxville, through the Acree Research Chair of Medicine endowment had no involvement in the design or performance of the study, writing of the manuscript, or the decision to submit the manuscript for publication.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

The study protocol was approved by the IACUC of the University of Tennessee, Knoxville (protocol number 2283) and performed in compliance with "The Guide for the Care and Use of Laboratory Animals" in laboratory animal facilities that are AAALAC certified and exceed NIH standards of care.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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