Effects of oral administration of controlled-ileal-release budesonide and assessment of pituitary-adrenocortical axis suppression in clinically normal dogs

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Objective—To evaluate the effects of oral administration of controlled-ileal-release (CIR) budesonide on the pituitary-adrenal axis in dogs with a normal gastrointestinal mucosal barrier.

Animals—10 healthy dogs.

Procedures—5 dogs received CIR budesonide orally once daily for days 1 through 28, and 5 dogs received placebo. Treatment group dogs that weighed < 18 kg received 2 mg of CIR budesonide; treatment group dogs that weighed ≥ 18 kg received 3 mg of CIR budesonide. In the treatment and placebo groups, there were 3 and 2 dogs, respectively, that weighed > 18 kg. Plasma cortisol concentration before and after ACTH stimulation, basal plasma endogenous ACTH concentration, and body weight were measured on days 0, 7, 14, 21, 28, and 35. Serum biochemical analysis, CBC determination, and urinalysis were performed on days 0, 28, and 35. On days 7, 14, and 21, serum ALP and ALT activities, serum glucose concentration, and urine specific gravity were obtained in lieu of a full hematologic evaluation and urinalysis.

Results—Basal and post-ACTH stimulation plasma cortisol concentrations and plasma endogenous ACTH concentration were significantly suppressed by treatment. No other variables were altered over the course of the study.

Conclusions and Clinical Relevance—Budesonide suppresses pituitary-adrenal function in dogs with normal gastrointestinal integrity, whereas other variables often affected by glucocorticoids were not altered by a 4-week treatment course. Budesonide may be a good alternative to traditional cortico-steroids if used short-term for acute exacerbations of inflammatory bowel disease. (*Am J Vet Res* 2006;67:1173–1178)

Abbreviations

IBD	Inflammatory bowel disease
CIR	Controlled-ileal-release
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
eACTH	Endogenous ACTH

Inflammatory bowel disease in dogs encompasses many disorders of the gastrointestinal tract in which recurrence of clinical signs occurs, such as vomiting, diarrhea, anorexia, and weight loss, and histologic evidence of intestinal inflammation is found.[1] These diseases are considered idiopathic, as no definitive etiologic agents or pathophysiologic processes have been elucidated, but the pathogenesis likely results from an exaggerated immune response by the intestinal mucosa to local antigens.[2] As a result of this presumed immunopathogenesis, glucocorticoids have long been the mainstay for treating IBD in dogs and humans. As in humans, adverse effects of glucocorticoids in dogs can be considerable and often create quality-of-life issues for owners and pets alike. Short-term treatment of dogs often produces severe polyuria and polydipsia and a ravenous appetite, with increased incidence of house soiling, inappropriate foraging for food, or food aggression. Long-term glucocorticoid administration can lead to obesity, muscle atrophy and weakness, ligament rupture, and diabetes mellitus.

As a result of the adverse systemic effects of glucocorticoids, interest has developed recently in finding alternatives to traditional corticosteroid treatments for IBD. The glucocorticoid budesonide was developed for humans with regional enteritis (ie, Crohn's disease),[3,4] an IBD affecting the ileum and proximal portion of the large intestine. Because budesonide undergoes extensive first-pass hepatic metabolism in humans, with 90% of systemically available drug converted to less active metabolites, it theoretically has increased topical activity with minimal systemic effects.[3,4] In humans, budesonide is as effective as prednisolone for controlling intestinal inflammation while decreasing adverse effects and causing less adrenal gland suppression.[4-7]

Budesonide has been used in veterinary medicine to treat patients with IBD, with anecdotal evidence of success. To our knowledge, no studies on the pharmacokinetics, safety, or efficacy of budesonide have been performed in dogs. To date, only a single veterinary study[8] on budesonide has been performed, which assessed the effects of oral administration of powder-based budesonide on the pituitary-adrenal axis in client-owned dogs with active IBD. Basal and post-

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ACTH stimulation serum cortisol concentrations were significantly suppressed in treated dogs. One possible reason for the observed adrenal gland suppression in these dogs is that the inflamed gastrointestinal mucosal layer allowed for greater budesonide absorption.

The goal of this study was to evaluate the effects of oral administration of CIR budesonide on the pituitary-adrenal axis in dogs with a normal gastrointestinal mucosal barrier. We chose this formulation because it is likely to be most readily available to veterinarians in the United States in the foreseeable future. We also monitored objective indicators of systemic effects of corticosteroids, such as serum ALP and ALT activities, serum glucose concentration, body weight, presence of microalbuminuria, and urine specific gravity. Because glucocorticoids can influence the leukogram, we also evaluated effects of budesonide on WBC counts, including total leukocyte, neutrophil, eosinophil, band neutrophil, and lymphocyte concentrations.

Materials and Methods

Animals—Ten sexually intact male dogs, aged 1 to 6 years, were selected from a pool of healthy laboratory dogs. Three dogs were purebred Beagles, and the others were mixed-breed Beagle or hound dogs. Spayed females were not available, and unspayed females were excluded from the study to eliminate the possible effects of estrus on the pituitary-adrenocortical axis as a confounding factor. Body weights ranged from 8.8 to 26.0 kg.

Dogs were judged to be healthy on the basis of physical examination findings, CBC determination, serum biochemical analysis, urinalysis, and results of heartworm occult antigen testing. All dogs were housed in standard university kennels and were fed adult maintenance kibble ad libitum. The Auburn University Institutional Animal Care and Use Committee approved the research protocol.

Experimental protocol—Dogs were matched on the basis of weight and assigned to the treatment (budesonide) or placebo group, with 5 dogs/group. Treatment group dogs that weighed < 18 kg received 2 mg of CIR budesonide,[a] PO, daily, and those that weighed ≥ 18 kg received 3 mg of budesonide, PO, daily. In the treatment and placebo groups, there were 3 and 2 dogs, respectively, that weighed > 18 kg. The 2-mg capsules were compounded from commercially available 3-mg capsules by estimated fractional distribution of coated granules into gelatin capsules. Placebo group dogs received an empty gelatin capsule daily. Medications were administered daily at 8 AM for 28 days (days 1 to 28), then discontinued for the remaining 7 days of the study (days 29 to 35).

Dogs were examined and tested weekly for the duration of the study. Blood samples for determination of pre- and post-ACTH stimulation plasma cortisol concentrations and basal plasma eACTH concentrations were collected on days 0, 7, 14, 21, 28, and 35. Serum biochemical analysis, CBC determination, and urinalyses were performed on days 0, 28, and 35. On days 7, 14, and 21, serum ALP and ALT activities, serum glucose concentration, and urine specific gravity were obtained in lieu of a full hematologic evaluation and urinalysis. Body weight was recorded and urine assayed for the presence of microalbuminuria on all test dates.

Assay procedures—Blood samples for CBC determination were collected via jugular or cephalic venipuncture into evacuated glass tubes containing EDTA. Blood for serum biochemical analysis and determination of serum ALP and ALT activities and glucose concentration was collected into anticoagulant-free glass tubes and allowed to clot for at least 30 minutes. Blood samples were centrifuged, and the serum was removed. Urine was obtained by free-catch collection, cystocentesis, or catheterization. Complete blood count determination, serum biochemical profile analysis or analysis of individual serum biochemical variables, and urinalyses were all performed on the day of sample collection.

Blood samples for basal cortisol concentration measurements were collected into glass EDTA-containing evacuated tubes. Cosyntropin[b] was administered IV (5 µg/kg) into a cephalic vein. One hour after injection, blood samples were again collected into EDTA-containing tubes for assay of post-ACTH stimulation plasma cortisol concentration. Blood for basal plasma eACTH concentration measurements was collected prior to cosyntropin administration into EDTA-containing glass tubes. The preservative, aprotinin, was immediately added to the sample.[9] All blood samples were centrifuged within 3 minutes of collection, and the plasma was separated and placed in polystyrene tubes. Samples were stored at –20°C until analysis as a single batch.

Cortisol samples were assayed in duplicate by use of a previously validated radioimmunoassay.[10,c] Samples for eACTH measurements were assayed singly by use of a previously validated immunoradiometric kit.[11,d] Microalbuminuria was measured by use of a commercially available kit.[e] The test was performed according to manufacturer's instructions.

Statistical analysis—For each variable, comparisons were made between the treatment and placebo group and between groups on each date related to baseline. Analysis was performed with a commercial program[f] by use of a repeated-measures ANOVA on ranks. If a significant difference was found, post hoc comparisons were made by use of the Student-Neuman-Keuls method. Significance was set at a value of $P < 0.05$.

Results

No significant difference existed between groups at baseline for any variable. In the budesonide-treated group, median basal plasma cortisol concentration was significantly ($P < 0.001$) suppressed, compared with baseline and the control group on days 7, 14, 21, and 28. By the last day of the study (day 35), treated dogs had not received budesonide for the previous 7 days and basal plasma cortisol concentration had returned to within reference range, with no significant difference from control or baseline values (**Figure 1**). No change in basal plasma cortisol concentration occurred in dogs receiving the placebo.

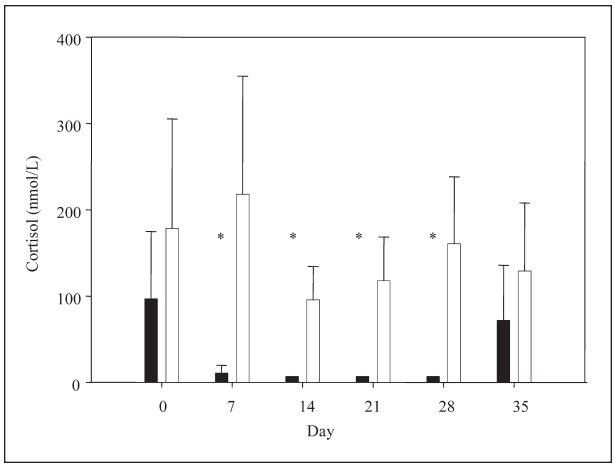

Figure 1—Mean ± SD basal plasma cortisol concentrations in dogs receiving budesonide (n = 5; solid bars) and placebo (5; open bars) on days 1 through 28. *Significantly ($P < 0.05$) different from control group and from baseline.

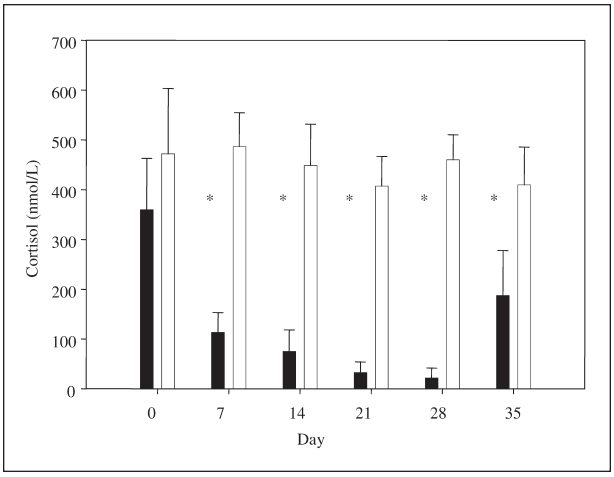

Figure 2—Mean ± SD post-ACTH stimulation plasma cortisol concentrations in dogs receiving budesonide (n = 5; solid bars) and placebo (5; open bars) on days 1 through 28. *See* Figure 1 for key.

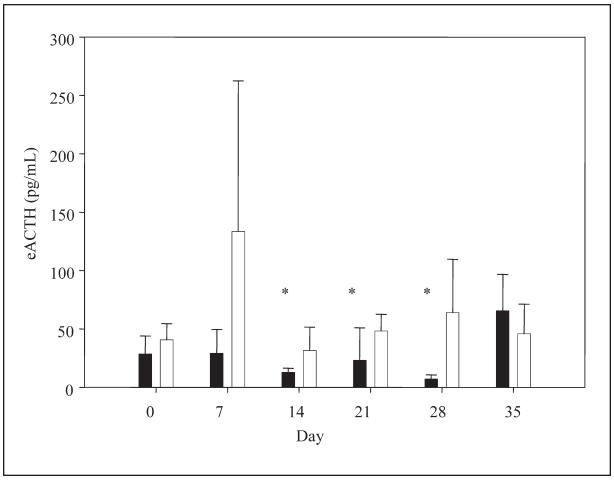

Figure 3—Mean ± SD basal plasma eACTH concentrations in dogs receiving budesonide (n = 5; solid bars) and placebo (5; open bars) on days 1 through 28. *See* Figure 1 for key.

In the budesonide-treated group, post-ACTH stimulation plasma cortisol concentration was significantly ($P < 0.001$) lower, compared with baseline and the control group on days 7, 14, 21, 28, and 35 (**Figure 2**). No change in post-ACTH stimulation plasma cortisol concentration occurred in dogs receiving a placebo. In the budesonide-treated group, plasma eACTH concentration was significantly ($P < 0.001$) decreased, compared with the control group and baseline on days 14, 21, and 28 (**Figure 3**). No change in plasma eACTH concentration occurred in dogs receiving the placebo.

No significant differences were found between groups or within groups for body weight, serum ALP and ALT activities, serum glucose concentration, urine specific gravity, total WBC count, blood lymphocyte or eosinophil concentrations, or blood lymphocyte-to-neutrophil ratios throughout the study. Serum ALP activity, in fact, was never greater than reference range values for any treated dog at any time during the study (**Figure 4**). No dog in either group consistently had microalbuminuria during the course of the study.

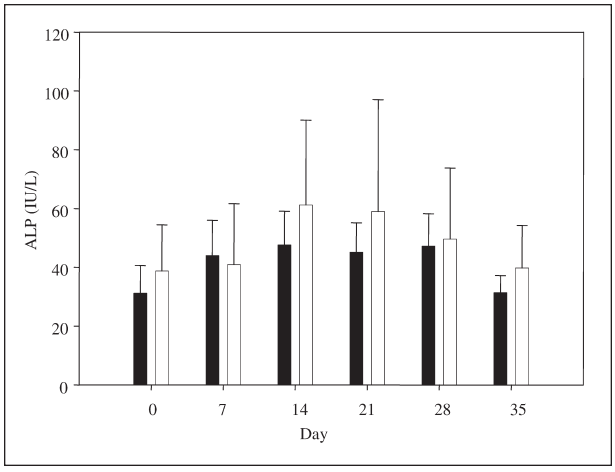

Figure 4—Mean ± SD serum ALP activities in dogs receiving budesonide (n = 5; solid bars) and placebo (5; open bars) on days 1 through 28.

Discussion

Budesonide is a nonhalogenated glucocorticoid that has been used in aerosol form since the early 1980s to treat asthma and rhinitis in humans.[12] More recently, forms for oral and rectal administration have been developed for treatment of IBD (ie, Crohn's disease) and ulcerative colitis, respectively.[3] The CIR capsule for oral administration contains coated budesonide microgranules designed to survive transit through the acid gastric environment and to dissolve at a pH of > 5.5, with maximal release in the ileum and proximal portion of the large intestine.[13]

After initial absorption from a mucosal surface, budesonide undergoes extensive first-pass metabolism in the liver in humans via the cytochrome P450 system.[14] Approximately 90% of systemically available budesonide is converted primarily to 16-α-hydroxyprednisolone and 6-β-hydroxybudesonide.[3,15] These metabolites have a tenth to a hundredth the activity of the parent budesonide[3] and are primarily excreted in the urine.[16] Budesonide has high water solubility and moderate lipophilicity, thus improving distribution to mucosal surfaces and penetration into local tissue cells. After intracellular uptake, budesonide is reversibly converted to highly lipophilic esters, which are stored in the cell. The esters are gradually hydrolyzed, thus releasing active budesonide and prolonging its anti-inflammatory effects.[15] The rapid metabolism of budesonide and concentrated local effects as a result of mucosal retention allow increased topical activity with low systemic availability.

In humans, the low systemic availability of budesonide theoretically provides an advantage over conventional corticosteroids such as hydrocortisone and prednisolone because budesonide causes fewer adverse effects and less adrenocortical suppression with comparable efficacy.[3,6,17] Pituitary-adrenal axis suppression does occur, however, to some degree. In adult humans treated with an appropriate dose of budesonide, 69% had an impaired response to ACTH stimulation after 8 weeks of treatment. However, in this same group of patients, the frequency of other adverse effects was no different, compared with placebo.[4]

In dogs, glucocorticoid-induced suppression of the pituitary-adrenal axis has been demonstrated for multiple corticosteroid formulations and routes of administration, including topical and ophthalmic.[18-26] To our knowledge, the only veterinary study[8] published on budesonide was a noncontrolled, nonrandomized report of 6 dogs with active IBD and histopathologically proven disease. Suppression of the pituitary-adrenal axis was assessed in response to the pure powder-based formulation of budesonide, compounded at a dose of 3 mg/m^2, which is roughly equivalent to 1 mg/10.7 kg. All 6 dogs in that study had substantial adrenocortical suppression after 30 days of treatment. Mean post-ACTH stimulation serum cortisol concentration decreased from 348 nmol/L (12.6 µg/dL) to 83 nmol/L (3.0 µg/dL).[8] The systemic effect may have been the result of increased drug absorption through a compromised intestinal mucosa. As drugs have a higher absorption at sites of inflammation or increased blood flow,[27] dogs with active intestinal mucosal inflammation might have increased absorption of budesonide. Thus, one of the goals in our study was to determine whether dogs with a healthy intestinal mucosal barrier would have evidence of substantial absorption and pituitary-adrenal axis suppression.

Despite having a normal gastrointestinal mucosa, budesonide-treated dogs in our study had significant pituitary-adrenal axis suppression as early as day 7. Suppression of post-ACTH stimulation plasma cortisol concentration continued through day 35, 7 days after discontinuation of budesonide, although basal cortisol concentration had returned to normal by that time. Dogs receiving an anti-inflammatory dose of prednisone (1.1 mg/kg/d) orally had significant adrenal gland suppression after 2 weeks of treatment.[28] Although direct comparisons of budesonide to prednisone were not made in our study, it is interesting to note that in another study,[28] prednisone and budesonide caused rapid suppression, even though prednisone theoretically has a much higher bioavailability of 50% to 90%.[28] Budesonide, however, has a 15-fold higher affinity for the glucocorticoid receptor than prednisolone in rats.[29,30] Because glucocorticoid receptors are found in high concentrations in canine pituitary glands,[28] perhaps budesonide binds rapidly and strongly to these receptors overcoming a putative lower systemic availability.

Budesonide-treated dogs also had significantly decreased plasma eACTH concentration, compared with baseline or placebo, indicating direct pituitary suppression by budesonide. Interestingly, plasma eACTH concentration was not significantly suppressed until day 14 of treatment, when plasma cortisol concentration was diminished by day 7. Typically, decreases in plasma eACTH concentration precede adrenocortical suppression. As ACTH secretion is episodic, basal blood samples may have been drawn by chance on day 7, when eACTH concentrations were relatively high.

No significant changes in serum ALP and ALT activities were found between treatment and placebo groups at any time, nor did significant changes occur in either group, compared with baseline. It is well-known that exogenous glucocorticoids can cause increases in serum ALP and ALT activities in dogs,[31-38] although these effects tend to be dependent on the dose and duration of administration. In Beagles treated daily with 4.4 mg/kg of prednisone IM and an immunosuppressive dose of glucocorticoids, serum ALP activity was significantly increased by day 2 and ALT activity by day 3.[31] In contrast, dogs receiving an anti-inflammatory dose of prednisone (1.1 mg/kg) orally for 35 days did not have significant increases in mean total serum ALP or ALT activities, despite pituitary-adrenal axis suppression being evident at 2 and 4 weeks. Some individual dogs, however, had values that were greater than the reference range after 4 weeks.[39] In budesonide-treated dogs, mean serum ALP and ALT activities were unchanged throughout our study. In fact, neither variable was greater than the reference range in any treated dog at any time. Thus, the effects of exogenous glucocorticoids on serum ALP and ALT activities do not correlate with the degree of pituitary-adrenal axis suppression.

No changes in body weight or urine specific gravity were found over time in either group of dogs. Indeed, apart from 1 dog on a single occasion, all treated dogs had a urine specific gravity of > 1.025 on all test dates. Microalbuminuria was not consistently induced in any dog during our study. Naturally occurring hyperadrenocorticism[40,41] and high doses of prednisone (2.2 mg/kg, PO, q 12 h for 42 days)[42] can cause increases in the urine protein-to-creatinine ratio, which signifies a greater degree of proteinuria than does microalbuminuria. Likely, the dose and duration of glucocorticoid affect the severity and rapidity of onset of proteinuria.

Tumulty et al[8] evaluated subjective variables (water intake, micturition frequency, and appetite) commonly affected by traditional glucocorticoids in their budesonide-treated dogs and found no changes. Interestingly, humans that developed an impaired response to ACTH stimulation after 8 weeks of budesonide treatment also did not have other substantial adverse effects,[4] indicating that in dogs and humans, the pituitary-adrenal axis is the most sensitive to glucocorticoid effects and is suppressed before other systemic changes occur.

Glucocorticoids commonly affect WBC concentrations, and their administration is typically associated with mature neutrophilia, lymphopenia, eosinopenia, and monocytosis.[39] In dogs receiving an anti-inflammatory dose of prednisone daily, eosinophil and lymphocyte counts were the most sensitive to treatment and decreased significantly within 2 weeks of administration.[39] No changes in WBC counts were found in budesonide-treated dogs, indicating that effects on the leukogram are minimal at the dose and duration of treatment in our study.

In humans, budesonide absorption appears to be independent of intestinal disease. In a pharmacokinetic study,[43] no difference existed in systemic availability of drug between children with mild versus severe Crohn's disease. In addition, budesonide has approximately 9% to 12% systemic availability in patients with Crohn's disease[43] and healthy volunteers.[16,30] Our study found pituitary-adrenal axis suppression in dogs with normal gastrointestinal mucosal integrity, indicating

that systemic budesonide absorption occurs in the absence of intestinal disease in dogs as well.

A limitation of our study was the duration of treatment. Although pituitary-adrenal axis suppression was evident early in the course of treatment, objective variables that reflect glucocorticoid administration in dogs were not affected after 28 days of receiving budesonide. Similarly, after 30 days of treatment, none of the 6 dogs in the study by Tumulty et al[8] had changes in objective (eg, ALP and urine specific gravity) or subjective (eg, water intake, micturition frequency, and appetite) variables commonly affected by traditional glucocorticoids. Perhaps longer treatment of the dogs in our study would have eventually led to changes in some of the other measured variables. More prolonged pituitary-adrenal axis suppression might also have led to clinical signs of adrenocortical deficiency upon discontinuation.

Another study limitation was budesonide dose selection. To our knowledge, no pharmacokinetic studies have been performed in dogs. Therefore, the dose used was extrapolated from widely available anecdotal dosages. Doses used in our study might not necessarily provide successful remission of IBD. In humans, pharmacokinetics and systemic availability of oral budesonide are similar for children that are > 20 kg and adults receiving 9 mg/patient/d,[43] a dose generally accepted as the lowest effective dose for inducing remission of Crohn's disease.[4] Thus, dosing of budesonide may be independent of weight in humans, but dogs have much greater interindividual variability in body weight than occurs between adult humans and noninfant children. Humans receiving 3 mg of budesonide did not have significantly different remission rates of Crohn's disease, compared with placebo.[4] If the pharmacokinetics of budesonide in dogs are similar to humans, the dose selection of a maximum of 3 mg for any dog > 18 kg may be low. If higher doses are needed for efficacy, adverse effects of budesonide other than pituitary-adrenal axis suppression may be found earlier in the course of treatment.

Although pituitary-adrenal axis suppression did occur in budesonide-treated dogs, clinical evidence of adrenal gland deficiency after discontinuation was not evident and no objective adverse effects of treatment were found. Therefore, budesonide may still be a good alternative to traditional corticosteroids if used short-term for acute exacerbations of IBD. Further studies on the efficacy, pharmacokinetics, and intestinal delivery of budesonide are warranted to establish its clinical usefulness in veterinary medicine.

a. Entocort EC, AstraZeneca, Wilmington, Del.
b. Cortrosyn, Amphastar, Rancho Cucamonga, Calif.
c. Coat-a-Count cortisol assay, Diagnostic Products Corp, Los Angeles, Calif.
d. ACTH assay, Nichols Institute, San Clemente, Calif.
e. ERD-HealthScreen canine urine test, Heska Corp, Fort Collins, Colo.
f. SigmaStat for Windows, version 1.0, Jandel Scientific, SPSS Inc, Chicago, Ill.

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