Plasma concentrations and therapeutic effects of budesonide in dogs with inflammatory bowel disease

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Objective—To evaluate the pharmacokinetics and clinical efficacy of budesonide in dogs with inflammatory bowel disease (IBD).

Animals—11 dogs (mean ± SD age, 5.7 ± 3.9 years; various breeds and body weights) with moderate or severe IBD.

Procedures—Each dog received a controlled-release formulation of budesonide (3 mg/m², PO, q 24 h) for 30 days (first day of administration was day 1). The concentration of budesonide and its metabolite (16-α-hydroxyprogrenolone) was measured via liquid chromatography–tandem mass spectrometry in plasma and urine samples obtained on days 1 and 8 of treatment. On those days, plasma samples were obtained before the daily budesonide administration and 0.5, 1, 2, 4, and 7 hours after drug administration, whereas urine samples were obtained after collection of the last blood sample. A clinical evaluation was performed on the dogs before onset of drug administration and on days 20 and 30 after start of drug administration.

Results—The highest plasma concentration of budesonide and 16-α-hydroxyprogrenolone on day 1 was detected at 1 hour and at 2 hours after drug administration, respectively. After standardization on the basis of specific gravity, the ratio between urinary concentrations of budesonide and 16-α-hydroxyprogrenolone was 0.006 and 0.012 on days 1 and 8, respectively. The clinical response was adequate in 8 of 11 dogs.

Conclusions and Clinical Relevance—Budesonide was rapidly absorbed and metabolized in dogs with IBD. The drug gradually accumulated, and there was an adequate therapeutic response and no adverse effects. (Am J Vet Res 2013;74:78–83)
Adverse effects of corticosteroids are polyuria, polydipsia, increased appetite, obesity, muscle atrophy, weakness, diabetes mellitus, and susceptibility to infections. To limit these effects, budesonide was introduced in human medicine for the treatment of active Crohn’s disease and IBD. It is a nonhalogenated corticosteroid with a high affinity for glucocorticoid receptors, high hepatic clearance, and high local and low systemic activity, compared with other glucocorticoids.5,10

The interest in veterinary medicine regarding budesonide is related to the treatment of various forms of IBD in dogs that are intolerant to prednisone or dexamethasone. In these patients, a drug able to combine potent local activity with a low number of systemic effects could be useful.11

Although it has been used for many years in veterinary medicine, budesonide has not been fully evaluated in dogs, and knowledge is lacking regarding its kinetics and the main metabolites (16-α-hydroxybudesonide and 6β-hydroxybudesonide) formed in the liver. In humans, 90% of the parent drug is transformed into these 2 metabolites.12 In humans, the pharmacological activity of these metabolites, which are excreted via the urine and feces, is extremely low (≤ 1%), compared with that of budesonide.13 The purpose of the study reported here was to evaluate the pharmacokinetics of a commercial formulation of budesonide in dogs with naturally occurring IBD by analyzing the concentrations of budesonide and one of its metabolites (16-α-hydroxybudesonide) before and during oral administration and to evaluate the clinical efficacy of the drug.

Materials and Methods

Animals—Eleven client-owned dogs (6 males [5 sexually intact and 1 neutered] and 5 females [4 sexually intact and 1 spayed]) with a mean ± SD age of 5.7 ± 3.9 years were enrolled in a prospective, uncontrolled clinical trial that was conducted over a 30-day period. Dogs were of various breeds (4 mixed-breed dogs, 2 Boxers, 1 Rottweiler, 1 Dogue de Bordeaux, 1 Bulldog, 1 German Shepherd Dog, and 1 Cane Corso) and with body weights ranging from 15 to 48 kg (mean, 29.1 ± 11 kg). All owners provided written informed consent prior to enrollment of the dogs. The study was approved by the Scientific Ethics Committee for Experimentation on Animals of Alma Mater Studiorum–University of Bologna.

Inclusion criteria included a diagnosis of moderate or moderate-to-severe IBD. Inflammatory bowel disease was classified as proposed elsewhere.5,13,14 For the 11 dogs of the study, a diagnosis of IBD was made on the basis of persistent or recurrent gastrointestinal signs (11/11), weight loss (8/11), reduction of serum folate concentration (7/11), reduction of serum cobalamin concentration (6/11), increased concentrations of C-reactive protein (9/11), ultrasonographic patterns of bowel lesions (wall thickening, hyperchoeic speckles, and hyperchoeic striations; 10/10), and the results of histologic examination of intestinal biopsy specimens acquired via gastrointestinal endoscopy that revealed moderate (2/11) or severe (9/11) lymphocytic-plasmacytic enteritis. Other possible causes (eg, exocrine pancreatic insufficiency, infectious agents, or neoplasia) of the clinical signs were ruled out. Dogs that had received anti-inflammatory drugs within the 6 months preceding the study were excluded.

Before enrollment, each dog received an anthelmintic (fenbendazole; 30 mg/kg, PO, q 24 h for 5 days), was subjected to a dietetic trial for at least 3 weeks, and was treated with an antimicrobial (metronidazole; 10 mg/kg, PO, q 12 h) for at least 2 weeks. The response to treatment in all dogs was moderate to poor. Dogs with folate or cobalamin deficiency were treated via oral or parenteral administration, respectively. A CIBDAI was calculated for each dog prior to the study.

Study design—After histologic examination of biopsy specimens, each dog was treated with a commercially available controlled-release formulation of budesonide (3 mg/m², PO, q 24 h). The treatment consisted of capsules containing budesonide that were coated with a cationic copolymer based on dimethylaminoethyl methacrylate, butyl methacrylate, and methyl methacrylate to enable them to dissolve in the duodenum at pH > 5 rather than in the gastric environment.

The treatment was administrated once daily (9:00 AM) for 30 days (first day of treatment was designated as day 1). Plasma and urine concentrations of budesonide and 16-α-hydroxybudesonide were evaluated on days 1 and 8 of treatment. On both days, blood samples were collected into EDTA-containing tubes immediately before the daily budesonide administration (time 0) and 0.5, 1, 2, 4, and 7 hours after drug administration. Plasma was harvested and stored at −20°C for subsequent determination of the concentration of budesonide and 16-α-hydroxybudesonide.

Immediately after collection of the final blood sample on days 1 and 8, cystocentesis was performed to obtain a urine sample from each dog. The specific gravity of the urine was immediately evaluated with a refractometer. The urine samples then were centrifuged and the supernatant harvested and stored at −20°C until budesonide and 16-α-hydroxybudesonide were extracted.

A CIBDAI was calculated for each dog before and on day 20 after the start of budesonide administration. A clinical examination of each dog was performed before and on days 20 and 30 after the start of budesonide administration.

Analysis of plasma and urine samples—Budesonide, betamethasone, and 16-α-hydroxybudesonide standards were purchased. Analyte concentrations were determined via liquid chromatography–tandem mass spectrometry. The liquid chromatograph4 consisted of a quaternary pump, solvent degasser, auto sampler, and column heater. The mass spectrometer4 was a triple quadrupole instrument equipped with a multimode ionization source.

Plasma and urine samples were initially subjected to solid-phase extraction.6 After the addition of betamethasone as the internal standard, plasma samples (0.5 mL) were loaded onto activated cartridges. The cartridges were washed with a solution of methanol and water (5 parts methanol:95 parts water), and the analytes were eluted with methanol. Eluates were evaporated and dissolved in a 100-μL mixture of water,
acetonitrile, and formic acid (30 parts water:50 parts acetonitrile; and 0.1% formic acid); then, 10 µL of this solution was injected into the liquid chromatography–tandem mass spectrometry system. Chromatographic separation was achieved via reverse-phase chromatography with gradient elution. Mobile phase A comprised water containing 0.1% formic acid, and mobile phase B comprised acetonitrile containing 0.1% formic acid. The mass spectrometer interface was an electrospray ionization source operating in positive ion mode. The mass spectrometer was in a selected reaction monitoring mode and monitored 2 specific metabolites for each compound.

Statistical analysis—Plasma and urinary concentrations of budesonide and 16-α-hydroxyprednisolone were reported as mean ± SD. Mean values were compared via an ANOVA for repeated measures to examine differences in the plasma concentration of budesonide and 16-α-hydroxyprednisolone at various times throughout the study, followed by a post hoc Fisher least significant difference test, when appropriate. The CIBDAI values obtained before and after treatment with budesonide for 20 days were analyzed via a Student t test for paired data. Statistical software was used for all analyses. Values were considered significant at P < 0.05.

Results

The analytic method measured the 2 analytes with limits of detection of 0.10 ng/mL for budesonide and 0.20 ng/mL for 16-α-hydroxyprednisolone in plasma and 0.25 ng/mL for budesonide and 0.20 ng/mL for 16-α-hydroxyprednisolone in urine (Figure 1). Any measurements below the limit of detection were reported as 0.

Mean ± SD and significant differences in plasma concentrations of budesonide and 16-α-hydroxyprednisolone between samples obtained before drug administration and during the next 7 hours on days 1 and 8 of treatment were determined (Table 1). Mean ± SD urinary concentrations of the 2 analytes were examined in only 9 of 11 dogs; urinary concentrations standardized on the basis of a urinary specific gravity of 1.020 were 0.67 ± 1.42 ng/mL for budesonide and 119.94 ± 195.67 ng/mL for 16-α-hydroxyprednisolone on day 1 and 1.00 ± 1.50 ng/mL for budesonide and 86.97 ± 113.86 ng/mL for 16-α-hydroxyprednisolone on day 8.

A rapid increase in the plasma concentration of budesonide was detected on day 1, with the highest mean ± SD daily concentration (0.96 ± 0.52 ng/mL) at 1 hour after drug administration, whereas the mean maximum value for both days of plasma analysis (0.56 ± 0.37 ng/mL) was reached at 4 hours on day 8. The mean maximum plasma concentration of 16-α-hydroxyprednisolone throughout the entire period (1.64 ± 2.20 ng/mL) was detected at 2 hours after drug administration on day 1. Mean ± SD plasma concentrations of budesonide and 16-α-hydroxyprednisolone were > 0 (0.21 ± 0.26 ng/mL and 0.26 ± 0.61 ng/mL, respectively) before the daily drug administration on day 8, which indicated drug accumulation during the treatment period.

Plasma concentrations of budesonide and 16-α-hydroxyprednisolone in 1 dog were below the limit of detection in all plasma samples obtained on day 8, whereas another dog had 16-α-hydroxyprednisolone concentrations that were below the limit of detection in all plasma samples obtained on day 1. In contrast, budesonide or 16-α-hydroxyprednisolone was detected in urine samples obtained on those same days from both of these dogs. The budesonide and 16-α-hydroxyprednisolone concentrations in the urinary sample obtained on day 8 from the former dog were 1.34 and 55.55 ng/mL, respectively, whereas the 16-α-hydroxyprednisolone concentration in the urinary sample obtained from the latter dog on day 1 was 12.71 ng/mL.

The clinical evaluation on day 20 after start of drug administration indicated improvement, with a significant (P < 0.001)
decrease in the mean ± SD value for the CIBDAI (mean ± SD CIBDAI value at enrollment, 7.4 ± 3.1; mean CIBDAI value at day 20, 2.3 ± 2.1). However, at day 30 after the start of drug administration, 3 of 11 dogs did not have an adequate response to the drug and had evidence of large-bowel diarrhea. No episodes of polyuria, polydipsia, polyphagia, panting, a large abdomen, or hair loss were reported by the owners during the administration of budesonide for the 30 days of the study.

Discussion

Budesonide is a nonhalogenated glucocorticoid that has been used for several years in humans for the treatment of asthma, allergic rhinitis, and immunemediated diseases of the bowel (ie, Crohn’s disease or IBD). Budesonide has powerful local anti-inflammatory effects with little systemic involvement.

Results of a study in human medicine suggested there was an increase in budesonide absorption from inflamed bowels and an accumulation of budesonide in cells of the intestinal wall undergoing an esterification reaction with fatty acids. Conjugates formed in such a manner are inactive, have a low affinity or no affinity for glucocorticoid receptors, and represent a form of storage that can be released through a reversible process of ester hydrolysis by intracellular lipase. The active form is transported to the liver; in humans, 90% of budesonide is converted into 2 inactive metabolites (16-α-hydroxybudesonide and 6-β-hydroxybudesonide) in the liver, and little budesonide passes through the liver into the bloodstream, which means that there is minimal exposure to the drug’s corticosteroid effects. The mechanism of action of budesonide depends on linkage to a specific intracellular glucocorticoid receptor, for which budesonide has extremely high affinity (when applied topically, up to 200 times as great as that of hydrocortisone) and systemic bioavailability of only 10% after oral administration. It is interesting that compared with the broad use of this drug in humans, studies on the use of this drug in dogs are rare and have included the use of this compound in pneumology, dermatology, and gastroenterology.

The choice for use of a commercial product instead of pure budesonide was motivated by the desire to examine the behavior of a commercial drug that is used in dogs, rather than effects of the pure drug substance. Moreover, the choice of a controlled-release formulation was deemed necessary because many forms of IBD in dogs specifically involve the distal aspect of the jejunum and ileum.

However, it is important to mention that until now, no studies have established a single dose of budesonide for use in dogs. For example, orally administered doses suggested in previous studies of canine gastroenterology include 9 mg/dog, 3 mg/m2, and 2 mg for dogs weighing <18 kg and 3 mg for dogs weighing ≥18 kg. Because of the different body weights of the dogs enrolled in the present study, we selected a dose of 3 mg/m2. A pharmacokinetic analysis of the results for the present study indicated rapid absorption and metabolism of budesonide in dogs, with detectable plasma concentrations of budesonide and 16-α-hydroxybudesonide in the first sample (30 minutes after administration) in 5 of 11 and 3 of 11 dogs, respectively, whereas budesonide was detectable in the plasma of 9 of 11 dogs and 16-α-hydroxybudesonide was detectable in the plasma of 6 of 11 dogs at 1 hour after administration. In addition, the highest mean budesonide plasma concentration on day 1 was for the sample obtained 1 hour after drug administration.

Results of the present study differed from those of a similar study in which investigators used only healthy Beagles and a dose of 9 mg/dog. In that study, investigators determined that the mean ± SD maximum concentration of budesonide was reached 3.5 ± 3.3 hours after a single oral administration. Despite the differences between that study and the present study, it can be suggested that the absorption of budesonide in dogs with IBD is more rapid than it is in healthy dogs. The possibility that an inflamed bowel results in faster uptake of the drug was also reported in rats. In that study, investigators found an increased (but not significantly different) concentration of budesonide in inflamed ileal mucosa, compared with the concentration in noninflamed ileal mucosa. In addition, in another study performed in healthy human volunteers, a mean absorption time of 6 hours was reported, which is slower than the time detected in the dogs with IBD in the study reported here.

When we analyzed results of the present study, it was interesting to find that there was a gradual accumulation of budesonide and 16-α-hydroxybudesonide during the first 8 days of treatment, as indicated by mean ± SD plasma concentrations of 0.21 ± 0.26 ng/mL for budesonide and 0.26 ± 0.61 ng/mL for 16-α-hydroxybudesonide at time 0 on day 8. Similar to results for a study conducted on humans, this could have been attributable to biotransformation of budesonide in intestinal cells by an enzymatic isomerase cytochrome P450 3A4 with enteric cell deposits of lipophilic intracellular fatty acid esters that remained in equilibrium with the active form, which constituted a form of storage.

Regarding excretion of the drug and its metabolites, a primarily renal route of excretion of the metabolites with a small proportion of the unaltered molecule has been confirmed in humans. This appears to be consistent with results for dogs, as indicated by the fact that the excretion of 16-α-hydroxybudesonide was greater than that of budesonide, with a mean ratio between urinary concentrations of budesonide and 16-α-hydroxybudesonide of 0.006 on day 1 and 0.012 on day 8 of treatment.

It is worth mentioning that, in 2 dogs, there was a seemingly contradictory behavior between the plasma concentrations of budesonide and its metabolite and their concentrations in urine samples. In particular, all plasma samples obtained from one of these dogs on day 8 had concentrations of budesonide and 16-α-hydroxybudesonide that were below the limit of detection; similarly, all plasma samples obtained from the other dog on day 1 had a concentration of 16-α-hydroxybudesonide that was lower than the limit of detection. Superficial analysis of these results may...
have attributed these extremely low plasma concentrations of budesonide and 16β-hydroxyprednisolone to a lack of administration, rather than to a lack of intestinal absorption or to abnormal metabolism of the drug; however, budesonide and 16β-hydroxyprednisolone were detected in the urine. This means that the drug was properly administered, absorbed, and metabolized, even though concentrations of it or its metabolite were not detected in the plasma.

Two hypotheses could account for this outcome. First, there is the possibility of esterification of the molecule, which would be transformed into a non-detectable compound during solid-phase extraction, as confirmed in a study in rats. In the present study, the detection of budesonide or 16β-hydroxyprednisolone (or both) in urine samples but not in plasma samples could be explained by subsequent de-esterification of budesonide or 16β-hydroxyprednisolone (or both) in the kidneys. On the other hand, this assumption hardly seems plausible because budesonide and 16β-hydroxyprednisolone were not detected in plasma samples obtained from only 2 of the 11 dogs and not on the same day of the study (one on day 1 and the other on day 8).

The second hypothesis is based on the detection limits of the analytic method used, whereby the plasma concentrations of budesonide and 16β-hydroxyprednisolone were less than the limits of detection and thus could not be detected before being concentrated and excreted by the kidneys.

Analysis of the clinical response of the dogs 20 days after the start of treatment revealed marked clinical improvement in the form of a significant reduction in CIBDAI values. However, after 30 days of treatment, 3 of 11 dogs did not have an adequate response to the drug and had evidence of large-bowel diarrhea. The explanation for this lack of a therapeutic response could be linked to the formulation of the drug, which consisted of coated granules to protect against dissolution in the gastric environment, with a matrix of ethyl cellulose with budesonide for release into the intestinal lumen in a time-dependent manner. Thus, this was a formulation designed to exert its effects in the ileum or ascending colon of humans; therefore, it is uncertain whether this drug was released in the correct anatomic site in dogs. In fact, it is possible that the main site of action of this drug is the small bowel in dogs, with less activity in the colon; this would explain the persistence of clinical signs related to inflammation of the large bowel.

Another possible explanation of the partial failure to respond to treatment with budesonide could be related to high P-glycoprotein expression in lymphocytes of the enteric lamina propria. P-glycoprotein is one of the main drug efflux pumps able to remove glucocorticoids from cells, which leads to low intracellular concentrations of corticosteroids and reduces clinical efficacy of treatment. It should be emphasized that regardless of the therapeutic response to budesonide, none of the dogs had polyuria, polydipsia, or other clinical signs compatible with iatrogenic hyperadrenocorticism at 30 days after the start of treatment.

It has been confirmed that although budesonide is largely catabolized, it can still have an inhibitory action on the pituitary-adrenal axis as a result of the extremely high affinity of the molecule for glucocorticoid receptors. In that study, investigators detected significant suppression of the pituitary-adrenal axis after treatment with budesonide for 30 days. However, it should be stressed that inhibition of the pituitary-adrenal axis was not associated with increases in the serum alanine phosphatase activity, a reduction in urine specific gravity, or signs of polyuria, polydipsia, or polyphagia (eg, iatrogenic hyperadrenocorticism).

Interestingly, humans who developed an impaired response to corticotrophin stimulation after budesonide treatment, even though the magnitude of hypothalamic-pituitary-adrenal suppression was modest and was less than that which would be expected from long-term administration of anti-inflammatory doses of conventional corticosteroids, did not have other substantial adverse effects. This indicates that in humans, similar to the situation in dogs, the pituitary-adrenal axis is the most sensitive to glucocorticoid effects and is suppressed before the onset of other systemic changes.

One limitation of the present study is the fact that the time period was too brief to enable us to evaluate the ability of the drug to adequately control IBD for a period >30 days. Thus, the possibility of any adverse effects with more prolonged administration is not known. However, even with this time limit, we confirmed that budesonide administered orally at 3 mg/m² appeared to provide an adequate therapeutic response without adverse effects in dogs affected by moderate or severe IBD, at least during the first month of treatment.

References