Evaluation of gastrointestinal permeability and mucosal absorptive capacity in dogs with chronic enteropathy

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Objective—To assess intestinal mucosal function by measuring permeability and absorptive capacity in dogs with chronic enteropathy (CE) before and after treatment and to determine whether those variables were correlated with clinical disease activity or histologic scoring of intestinal biopsy specimens.

Animals—29 dogs with CE.

Procedure—Dogs were designated as having diet-responsive CE or CE requiring glucocorticoid treatment. Severity of clinical signs was assessed by calculating the canine inflammatory bowel disease activity index (CIBDAI). Histologic severity of intestinal infiltration was assessed before and after 4 weeks of treatment in the diet-responsive group and before and after 10 weeks of treatment in the glucocorticoid group. Gastrointestinal permeability and mucosal absorptive capacity were assessed by use of intragastric administration of a solution containing lactulose, rhamnose, xylose, 3-O-methylglucose, and sucrose. Urine was collected 6 hours after administration of the sugar solution to determine urinary lactulose-to-rhamnose (L:R), xylose-to-methylglucose (X:M), and sucrose-to-methylglucose (S:M) ratios.

Results—Median CIBDAI scores decreased significantly in both groups of dogs after treatment. However, the median histologic grade of intestinal biopsy specimens did not change with treatment in either group. There were no significant differences in L:R, X:M, or S:M ratios before and after treatment in either group, and no significant correlations between L:R, X:M, or S:M ratios and CIBDAI or histologic scores.

Conclusions and Clinical Relevance—Results of tests for intestinal permeability and mucosal absorptive capacity were not useful indicators of clinical disease activity as assessed by the CIBDAI or the severity of infiltration as indicated by histologic evaluation. (Am J Vet Res 2006;67:479-483)

Chronic enteropathies are common conditions in dogs, and diet-responsive diarrhea and idiopathic inflammatory bowel disease are among the most important primary differential diagnoses. Determination of intestinal permeability and mucosal absorptive capacity is a noninvasive means of assessing intestinal damage in affected dogs. With this technique, a solution containing 1 or more marker molecules is administered orally and urinary excretion of the molecules is measured. In the past, radio-labeled EDTA (15Cr-EDTA) has been used as a marker to assess intestinal mucosal permeability in dogs, but more recently, methods involving determination of differential sugar absorption have been developed to overcome the practical limitations of working with radiochemicals.

Permeation of indigestible sugars occurs through small pores in the cell membrane or through leaky tight junctions in the mucosal epithelium. Therefore, intestinal permeability to large sugar molecules (eg, lactulose) is increased in animals with CE, compared with healthy animals. Small sugar molecules (eg, rhamnose or mannotol) normally cross the epithelium through pores in the cell membrane. In dogs with damaged mucosal epithelium, the decreased surface area leads to a decrease in permeability of the mucosal barrier to rhamnose. Sucrose is a large molecule that does not permeate healthy gastrointestinal mucosa intact but is metabolized by the intestinal brush border enzyme sucrase to fructose and glucose, which are subsequently absorbed via specific carriers in the intestinal mucosa. Intact sucrose molecules should not, therefore, be detected in the urine of healthy dogs after oral administration of a sucrose solution. To eliminate errors associated with individual variability in gastric emptying, intestinal transit time, or completeness of urine collection, recovery ratios for at least 2 types of sugar molecules may be calculated. Factors not related to the mucosa would be expected to affect the recovery of both sugars equally; therefore, only mucosal factors influence the ratio of recovery of 2 sugars. Formerly, these tests were cumbersome because urine had to be collected in a closed system over 6 to 24 hours. Recently, an abbreviated protocol has been used in which a single 10-mL sample of urine is obtained after 6 hours, making the test more applicable under clinical conditions.

In critically ill animals and in dogs in the acute stages of parvoviral infection, testing mucosal permeability and

| CE | Chronic enteropathies |
| L:R | Lactulose-to-rhamnose ratio |
| X:M | Xylose-to-methylglucose ratio |
| S:M | Sucrose-to-methylglucose ratio |
| CIBDAI | Canine inflammatory bowel disease activity index |
| DRD | Diet responsive disease |
| CRD | Corticosteroid responsive disease |
ability and absorption capacity by use of urinary L:R and X:M ratios has been useful for assessing mucosal damage associated with intestinal disease.\textsuperscript{12,15} In addition, sucrose permeability testing has been successfully used to measure gastric mucosal permeability in rats and dogs.\textsuperscript{14-16} However, to the authors’ knowledge, no recent studies have been conducted to assess the usefulness of the combined 5-sugar permeability and absorption test in a large number of dogs with CE. The objective of our study was to investigate changes in the results of these tests before and after treatment with corticosteroids or dietary modification in dogs with CE. In addition, the relationships between test results, clinical signs of disease, and severity of histologic abnormalities in intestinal mucosal specimens were evaluated.

**Materials and Methods**

**Animals**—Twenty-nine client-owned dogs of various breeds, age, and sex that had been referred to the Small Animal Teaching Hospital of the University of Bern for diagnostic endoscopy for evaluation of chronic diarrhea were recruited from 2002 to 2004. Some combination of altered attitude or demeanor, increased frequency of defecation, loose fecal consistency, weight loss, and reduced appetite had been observed in all dogs for several months at the time of enrollment into the study. Bacterial or parasitic diseases were eliminated as causes of CE by means of assays for fecal parasites and fecal bacterial culture prior to commencement of additional diagnostic testing.

Initial screening included a CBC, serum biochemical analysis, and urinalysis to exclude metabolic causes for CE. Serum trypsinlike immunoreactivity and serum concentrations of cobalamin and folate were measured at the laboratory of the Department of Pathology and Infectious Diseases at the Royal Veterinary College in London. In addition, dogs underwent transabdominal ultrasonographic examination to help exclude other causes of chronic diarrhea.

During physical examination, dogs were evaluated according to a described CIBDAI.\textsuperscript{17} Variables scored included attitude or level of activity, appetite, vomiting, fecal consistency, frequency of defecation, and weight loss. The composite score was used to differentiate among grades of disease severity as follows: 0 to 3, healthy to clinically unimportant signs of gastrointestinal disease; 4 to 5, mild signs of gastrointestinal disease; 6 to 8, moderately severe signs of gastrointestinal disease; and ≥ 9, severe signs of gastrointestinal disease.

Owners signed an informed consent form in which they agreed to their dogs’ participation in the study. All experimental procedures were approved by the Cantonal Committee for Animal Experimentation, Bern (Experiment No. 72/02), and by the Ethics Committee of the Veterinary Faculty, University of Bern, Switzerland. Dogs were admitted to the hospital, and food was withheld for 2 days in preparation for endoscopic examination, during which biopsy specimens of the duodenum and colon were collected. After the procedure, dogs returned home and were fed a novel-antigen diet consisting of salmon, trout, canola meal, and rice\textsuperscript{6} for 1 week, after which they were designated as having DRD or CRD on the basis of reexamination and rescoring results.

Dogs classified as having DRD had improvement in clinical signs after the week of dietary treatment and were fed the novel-antigen diet for 3 additional weeks (total feeding period, 4 weeks) before undergoing reexamination and repeat endoscopic imaging. Dogs classified as having CRD did not respond to treatment with the novel antigen diet after 1 week. Those dogs were given a tapering course of orally administered prednisolone as follows: 1 mg/kg twice daily for 10 days, 0.5 mg/kg twice daily for 10 days, 0.5 mg/kg once daily for 10 days, and then 0.5 mg/kg every other day for 10 days. This regimen was administered in addition to feeding the novel-antigen diet. Dogs were reexamined and underwent repeat endoscopic imaging after 10 weeks of treatment, at which time prednisolone treatment had been discontinued for 10 days.

During the second endoscopic exam, duodenal and colonic biopsy specimens were again collected. Ten biopsy specimens of the duodenum and colon for histologic examination were collected from each dog during both endoscopic examinations (ie, before and after the treatment period). Tissue samples were embedded in paraffin and slides were prepared and stained with H&E, prior to being evaluated by a board-certified pathologist according to published criteria.\textsuperscript{18}

For the 5-sugar permeability and absorption test, a solution containing 1 g of lactulose, 1 g of rhamnose, 1 g of xylose, 0.5 g of 3-O-methylglucose, and 4 g of sucrose/100 mL was administered by stomach tube on the day before the endoscopic procedure, after food had been withheld for 12 hours. Dogs that weighed < 10 kg received 100 mL of the sugar solution, dogs that weighed 10 to 20 kg received 200 mL, and dogs that weighed more than 20 kg received 400 mL. Dogs were allowed free time in a run to urinate prior to administration of the sugar solution. Manual expression of the bladder was also performed to ensure complete emptying. A 10-mL urine sample was collected 6 hours after administration of the sugar solution. Urine samples were transferred into tubes containing 10 µL of sodium azide and stored immediately at −80°C until analysis. The L:R, X:M, and SM ratios were determined by means of high performance liquid chromatography and pulsed amperometric detection at the Gastrointestinal Laboratory at Texas A&M University by use of a described method.\textsuperscript{19}

**Statistical analysis**—A 2-sample Mann-Whitney U or Wilcoxon rank sum test was used to evaluate data between the dogs with CRD and those with DRD, and a paired t test (eg, Wilcoxon signed rank test) was used to evaluate pre- and posttreatment results within each group. Correlations were performed by use of a Fisher exact or χ² test. Correlations were evaluated with the Spearman rank correlation test. Values of P < 0.05 were considered significant. Analyses were performed with commercially available statistical software.\textsuperscript{20}

**Results**

**Animals**—Results of initial tests (CBC, serum biochemical analyses, urinalysis, serum trypsinlike immunoreactivity, and transabdominal ultrasonographic examination) did not reveal any underlying infectious, exocrine pancreatic, neoplastic, or other extra gastrointestinal etiology for the diarrhea in any dogs.

Breeds represented in the DRD group were Golden Retriever (3 dogs), German Shepherd Dog (3), Labrador Retriever (2), Bernese Mountain Dog (2), mixed-breed dogs (2), Alaskan Malamute (1), Dachshund (1), Great Dane (1), Leonberger (1), Tervuren (1), Shi Tzu (1), and Whippet (1). The distribution of sex among dogs in the DRD group was 13 sexually intact males, 5 spayed females, and 1 sexually intact female. Mean ± SD age of dogs in the DRD group was 3.5 ± 2.14 years.
Breedes represented in the CRD group were mixed-breed dogs (3 dogs), Dachshund (2), Mastiff (1), German Shepherd Dog (1), Rottweiler (1), Shar Pei (1), and Yorkshire Terrier (1). Distribution of sex among dogs with CRD was 4 sexually intact males, 1 castrated male, 4 spayed females, and 1 sexually intact female. Mean ± SD age of dogs in the CRD group was 6.8 ± 2.63 years.

The median CIBDAI score for dogs at the time of initial examination at the teaching hospital was not significantly different between groups (dogs with DRD: median score, 6.7; dogs with CRD: median score, 7.6 [P = 0.13]). Clinical signs improved significantly during the treatment period in both groups (dogs with DRD: median score before treatment, 6.7; after treatment, 1.1 [P = 0.001]; dogs with CRD: median score before treatment, 7.6; after treatment, 3.4 [P = 0.02]). The CIBDAI score did not correlate with L:R, X:M, or S:R ratios or with histologic scores. No influence of sex on L:R, X:M, or S:M ratios was observed. Mean age was significantly lower in dogs with DRD than in dogs with CRD (3.5 vs 6.8 years, respectively; P = 0.003).

Histologic evaluations revealed mild to severe infiltration of gastric, duodenal, and colonic mucosa by immune cells consisting predominantly of lymphocytes and plasma cells, with or without eosinophils. Histologic scores did not change significantly after treatment in either group (dogs with DRD: histologic score of biopsy specimen from duodenum before treatment, 1.5; after treatment, 1.2 [P = 0.6]; histologic score for colon before treatment, 0.89; after treatment, 1.1 [P = 0.2]; dogs with CRD: histologic score of biopsy specimen from duodenum before treatment, 2.25; after treatment, 2.12 [P = 0.6]; histologic score for colon before treatment, 1.6; after treatment, 1.8 [P = 0.09]).

Mean serum cobalamin concentrations were not significantly different from reference range values before or after treatment in the DRD or CRD group (reference range, > 200 ng/L; mean ± SD cobalamin concentration in the DRD group before treatment, 497 ± 230 ng/L; after treatment, 509 ± 217 ng/L [P = 0.5]; mean ± SD cobalamin concentration in the CRD group before treatment, 417 ± 306 ng/L; after treatment, 398 ± 82 ng/L [P = 0.29]). In the DRD group, serum cobalamin concentrations were within reference range before and after treatment in all dogs. Of the 10 dogs with CRD, 4 had low (range, 100 to 190 ng/L) serum cobalamin concentrations before treatment, whereas 3 had low (range, 101 to 178 ng/L) serum cobalamin concentrations after treatment.

In the DRD group, mean serum folate concentrations were significantly higher after treatment than before treatment (mean ± SD folate concentration before treatment, 11.9 ± 7.1 µg/L; after treatment, 15.8 ± 9.6 µg/L [P = 0.01]; reference range, 7.1 to 14.4 µg/L), but there was no difference in serum folate concentrations before and after treatment in the CRD group (folate concentration before treatment, 11.2 ± 5.1 µg/L; after treatment, 12.5 ± 6.4 µg/L [P = 0.29]). In the DRD group, 2 of 19 dogs had low serum folate concentrations before treatment and 1 dog had low serum folate after treatment. One of 19 dogs had high serum folate concentration before treatment, and 2 had high serum folate concentrations after treatment. In the CRD group, 8 of 10 dogs had low serum folate concentrations before treatment and no dogs had low serum folate concentration after treatment. Five of the 10 dogs with CRD had high serum folate concentrations before treatment, and 7 of the 10 dogs had high serum folate concentrations after treatment.

L:R, X:M, and S:M Ratios—Lactulose-to-rhamnose, X:M, and S:M ratios were calculated before and after treatment were summarized (Figures 1 and 2). The L:R ratios were not significantly different before and after treatment in either group (DRD group: L:R ratio before treatment, 0.11; after treatment, 0.11 [P = 0.88]; reference range, 0.05 to 0.15 CRD group: L:R ratio before treatment, 0.09; after treatment, 0.1 [P = 0.57]). Also, L:R ratios were within reference range for 16 of the 19 dogs with DRD before treatment and for 16 dogs after treatment; among the dogs with...
CRD, L:R ratios were within reference range in 6 of 10 dogs before treatment and in 8 dogs after treatment. Xylose-to-methylglucose ratios were higher than reported reference ranges in both groups but did not change after treatment (DRD group: X:M before treatment, 1.13; after treatment, 1.98 \( P = 0.08 \); reference range, 0.40 to 0.59; CRD group: X:M before treatment, 0.8; after treatment, 0.8 \( P = 0.8 \)). Also, X:M ratios were greater than reference range values in 8 of 10 dogs with CRD before treatment and in 9 of 10 dogs after treatment. Of the 19 dogs with DRD, 16 had values greater than reference range before treatment and all 19 dogs had values greater than reference range after treatment. Among dogs with abnormal values for X:M, no significant change was detected in values before and after treatment \( (P = 0.6) \).

Sucrose-to-methylglucose ratios were greater in both groups of dogs, compared with reference range values, but did not change after treatment (DRD group: S:M ratio before treatment, 0.05; after treatment, 0.31 \( P = 0.8 \); CRD group: S:M before treatment, 1.004; after treatment, 0.998 \( P = 0.3 \) reference range, 0 to 0.03). Also, among dogs with CRD, S:M ratios were greater than reference values in 5 of 8 dogs before treatment and in 3 of 8 dogs after treatment. In dogs with DRD, 6 of 8 dogs had a high S:M ratio before treatment and 4 of 8 dogs had a high ratio after treatment. Among dogs with abnormal S:M values, no significant change was observed in values before and after treatment \( (P = 0.4) \). No correlations between L:R, X:M, or S:M ratios and CIBDAI or histologic scoring of intestinal biopsy specimens were detected.

**Discussion**
In the dogs of this study, tests for intestinal permeability and mucosal absorptive capacity were not useful indicators for estimating clinical disease activity as assessed by use of the CIBDAI score or the degree of bowel infiltration as assessed by histologic score. Dogs in the DRD group were significantly younger than those in the CRD group. This is consistent with previous findings, in which the range of ages in dogs with adverse food reactions was lower than the range in dogs for which treatment with corticosteroids or antimicrobials was needed. Gastrointestinal signs in the dogs with CE enrolled in the present study improved after treatment, as indicated by significantly lower CIBDAI scores. Histologic analyses revealed mucosal infiltration with lymphocytes and plasma cells, a finding that was in agreement with those of earlier studies. However, no significant correlations were detected between clinical status and histologic scores. Moreover, although the index of clinical disease activity decreased during treatment, there was no change in histologic scores of intestinal biopsy specimens after treatment. The usefulness of histologic evaluation as an indicator of disease severity has recently been questioned because no validated criteria exist that enable consistent interpretation of histologic changes in dogs with CE. In our study, an attempt was made to interpret intestinal biopsy specimens according to standardized published criteria and all analyses were performed by the same pathologist.

It is possible that histologic evaluation is not a valid method of assessing disease remission in dogs with CE.

Results of permeability and absorption testing have been used as a functional measure of mucosal damage in dogs with CE. Abnormal values for permeability and absorption in dogs with CE have been reported. There is a consensus among many veterinarians that certain dogs with CE respond favorably to antimicrobial treatment; the term antibiotic-responsive diarrhea is sometimes used as a result. In the present study, dogs were not treated with antimicrobials because most were referred to the teaching hospital after several attempts at treatment with metronidazole by the referring veterinarian. Quantitative bacterial culture of duodenal juice was not performed in our dogs, but cobalamin concentrations were low in only 4 of 10 dogs in the more severely affected (CRD) group. In dogs with DRD, serum folate concentrations were significantly higher after treatment, mostly because of values from a single dog that had a very high serum folate concentration (63 \( \mu \)g/L) after treatment. In addition, the mean serum folate concentration after treatment was only marginally higher than the reference range and was therefore not considered clinically important. These data suggest that our dogs did not have bacterial overgrowth in the small intestine and were therefore not comparable with dogs described in earlier studies.

Another reason for the differing results in permeability and absorption testing in dogs in the present study could be the difference in breed distribution, compared with that in earlier studies. For instance, in most reports, German Shepherd Dogs either composed a high percentage of study dogs or were the breed exclusively affected findings that were dissimilar to ours (4/29 dogs in our study were German Shepherd Dogs). German Shepherd Dogs have previously been described as predisposed to developing CE and may have skewed the results towards the more severely affected dogs in those studies. In Irish Setters, results of permeability and absorption tests have been used to predict sensitivity to gluten. In other studies, abnormalities have been described in healthy Beagles as well as in Beagles with the syndrome of small intestinal overgrowth, which has been characterized by higher-than-normal numbers of aerobic and anaerobic bacteria in the duodenal juice of dogs with CE. No Beagles or Irish Setters were included in the present study, which may be a reason for the normal L/R ratios we observed. However, authors of a recent report also described reference values for permeability and absorption in dogs with clinically active CE. In that study, only dogs with protein-losing enteropathy showed an increase in gamma globulin and hypoalbuminemia had abnormal L/R ratios. In the present study, only 1 dog had protein-losing enteropathy (serum albumin concentration at the time of initial examination, 1.4 g/L; reference range, 2.2 to 3.5 g/L). That dog had a high L/R ratio before treatment was initiated, but the ratio fell into reference range after treatment with prednisolone (L/R ratio before treatment, 0.46; after treatment, 0.1; reference range, 0.05 to 0.15). Changes in the recovery percentages for lactulose in the urine of dogs with acute parvoviral infection and in dogs with acute traumatic

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injury have been reported. It is possible that the dogs in the present study were not as severely ill as those described in other reports. However, median CIBDAI scores in dogs with CRD and DRD were 7.6 and 6.7, respectively, scores that are associated with moderate disease activity in dogs with CE.

Sucrose-to-methylglucose ratios were high in both groups of dogs, compared with reference range values, but did not change after treatment. Determination of urinary sucrose recovery rates is useful for assessment of gastric permeability in laboratory dogs with non-steroidal anti-inflammatory–induced gastropathy. Endoscopic examination of the stomach wall revealed normal findings in all dogs in our study, and histologic changes in the gastric mucosa were limited to mild-to-moderate infiltration with lymphocytes and plasma cells, consistent with mild clinical disease. Thus, the high S:M ratios detected before and after treatment in the present study may indicate that the test was sensitive enough to detect mild clinical disease of the stomach mucosa. An alternative explanation is that limitation in methylglucose absorption resulting from small intestinal infiltration increased the S:M ratio.

In conclusion, in this group of dogs with CE, determination of urinary L-R and X:M ratios as a test for intestinal permeability and mucosal absorptive capacity was not a useful indicator of disease severity, compared with calculation of CIBDAI scores or determination of infiltrative severity via histologic evaluation.

References


