Metabolic Consequences of Gut Dysbiosis in Dogs with IBD

Jan S. Suchodolski, MedVet, DrMedVet, PhD, DACVM, AGAF
Texas A&M University
College of Veterinary Medicine
Department of Small Animal Clinical Sciences
Gastrointestinal Laboratory
College Station, TX
Jsuchodolski@cvm.tamu.edu

Abstract

Various studies have evaluated the intestinal microbiota and functional changes in dogs and cats with intestinal disease. One example is dysbiosis-induced bile acids dysmetabolism, which plays a role in chronic gastrointestinal (GI) disease and potentially also in metabolic syndrome. Initial studies have provided more insights into how various treatments (e.g., antibiotics, fecal microbiota transplantation) are able to modulate the intestinal microbiota in dogs with acute and chronic enteropathies.

Glossary of Abbreviations
ABST: Apical Sodium-Bile Acid Transporter
BAD: Bile Acid Diarrhea
EPI: Exocrine Pancreatic Insufficiency
FMT: Fecal Microbial Transplantation
GI: Gastrointestinal
IBD: Inflammatory Bowel Disease
IBS: Irritable Bowel Syndrome
SCFA: Short-Chain Fatty Acids

Introduction

The intestinal microbiota is the collection of living microorganisms (bacteria, fungi, protozoa, viruses) in the GI tract. Molecular analysis has revealed that the GI microbiota of mammals is highly diverse.[1] It is estimated that the intestine harbors approximately 10 times more bacteria than the number of host cells in the body. This highly complex microbial ecosystem has a crucial impact on host health and disease. Gut microbes are useful to the host by fending off transient pathogens, aiding in digestion and energy harvest from the diet, providing nutrition for enterocytes, and playing an important role in development and stimulation of the host's immune system. For example, nutrient sources such as complex carbohydrates (e.g., starch, cellulose, pectin) are fermented by bacteria, resulting in the production of short-chain fatty acids (SCFA). These act as energy sources for the host, regulate intestinal motility, and are important growth factors for epithelial cells. SCFA also have direct anti-inflammatory properties through expansion of immunoregulatory lymphocytes. Other bacterially derived metabolites such as indole, a byproduct of tryptophan degradation, or secondary bile acids, also are immunomodulatory, thereby maintaining immune homeostasis and strengthening intestinal barrier function. These beneficial effects of the gut microbiota reach beyond the GI tract.

Microbiota and Metabolic Changes in GI Disease

Studies have associated alterations in the composition of the intestinal microbiota (dysbiosis) with chronic GI diseases of dogs and cats.[2,3] Historically, *Lactobacillus* and *Bifidobacterium* spp. were considered the most important beneficial bacterial groups in the intestine. However, recent studies have shown that members of Clostridium clusters XIVa and IV (Lachnospiraceae, *Ruminococcus* spp., *Faecalibacterium* spp., and *Roseburia* spp.) are more consistently depleted in humans and dogs with IBD and acute colitis, suggesting that these groups are highly important in maintaining intestinal homeostasis.[2] Due to the important role of microbial-derived metabolites for host health, it is important to understand the metabolic consequences of GI inflammation and dysbiosis. Several studies have evaluated selected metabolites that are altered in GI disease. Due to complex interactions between intestinal absorption and microbial metabolism, the exact cause for changes in serum concentrations of serum metabolites is often unknown, but a better understanding of the physiological pathways is helpful to potentially pinpoint specific diseases. An example for this approach is the measurement of serum concentrations of cobalamin and folate, two important markers for GI disease. Disorders that may affect serum cobalamin and/or folate concentrations include small intestinal inflammation, exocrine pancreatic insufficiency (EPI), and small intestinal dysbiosis (SIBO/ARD).[4] Due to different sites of absorption, specific changes in serum cobalamin and folate concentrations can yield information on the localization and, potentially, the severity of intestinal disease.

Changes in intestinal bile acid metabolism are implicated as an important factor in intestinal inflammation in human patients with inflammatory bowel disease (IBD) and *C. difficile* infection, and the therapeutic correction of altered bile acid concentrations leads to improvement of intestinal inflam-

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mation.[5,6] Unconjugated bile acids are toxic to epithelial cells, which may result in increased intestinal permeability. Furthermore, altered bile acid profiles lead to changes in fat absorption from the small intestine. Primary bile acids (cholic and chenodeoxycholic acid) are converted to secondary bile acids (deoxycholic and lithocholic acids) exclusively by the intestinal microbiota. Bacteria such as *Clostridium scindens* and *C. hiranonis* exhibit 7α-dehydroxylating activity, resulting in the 7α-dehydroxylation and biotransformation of the primary bile acids into the secondary bile acids. There is emerging evidence that altered bile acid metabolism leads to bile acid malabsorption and, therefore, is a frequent cause of diarrhea in humans.[6,7]

Bile acid diarrhea (BAD) can be due to various causes. Bile acid diarrhea Type 1 is caused by bile acid malabsorption that is secondary to ileal resection or inflammation. Type 2 is idiopathic bile acid malabsorption and is likely due to a defective feedback inhibition of hepatic bile acid synthesis from dysfunction of the ileal hormone fibroblast growth factor 19 (FGF19). Type 3 bile acid diarrhea occurs secondary to various GI diseases including small intestinal dysbiosis, radiation enteropathy, celiac disease, and chronic pancreatitis. It has been suggested that approximately 30% of human patients with irritable bowel syndrome (IBS) and 40% of patients with Crohn's disease have bile acid diarrhea. In humans with IBD, the destruction of apical sodium-bile acid transporter (ASBT) in the ileum leads to decreased reabsorption of bile acids in the small intestine, causing increases in primary bile acids in the colon that cause secretory diarrhea. Interestingly, the use of glucocorticoids induces the expression of ASBT in the small intestine, potentially reducing bile acid malabsorption and improving diarrhea.

Another cause of BAD could be dysbiosis. In a recent study in humans, IBD was associated with bile acid malabsorption.[5] Fecal primary bile acids were significantly increased in active IBD, whereas fecal secondary bile acid concentrations were significantly decreased in IBD compared to healthy controls.[5] This change in bile acid metabolism was linked to the dysbiosis that was present in patients with IBD, suggesting that dysbiosis leads to a decrease in bacterial species with 7α-dehydroxylation activity. Preliminary data from dogs with chronic enteropathy also suggest impaired bacterial conversion from primary to secondary bile acids.[8] Another study reported altered fecal bile acid profiles in dogs with chronic enteropathy when measured by gas chromatography coupled with mass spectrometry (GC-MS) (unpublished data). Fecal samples were collected from healthy dogs (n = 13) and dogs with chronic enteropathy (n = 13), with biopsy-confirmed inflammation. Significant decreases were observed in the secondary bile acids lithocholic acid and deoxycholic acid, with both being significantly decreased in the dogs with chronic enteropathy compared to healthy dogs. A significant correlation was observed between an increased dysbiosis index and bile acid dysmetabolism. This would suggest that the dysbiosis observed in chronic enteropathies also leads to a decrease in bacterial species with 7α-dehydroxylation activity, as seen in humans.

Another recent study evaluated bile acid malabsorption in dogs with chronic diarrhea.[9] The authors measured serum concentrations of 7α-hydroxy-4-cholesten-3-one (C4) as a potential marker of bile acid malabsorption. C4 is an intermediate in the biochemical synthesis of bile acids from cholesterol, and serum C4 concentrations reflect the activity of the bile acid synthetic pathway. An increase in serum C4 concentrations are indicative of bile acid malabsorption in humans with chronic diarrhea, and these patients typically respond well to bile acid sequestrants (e.g., cholestyramine).[6] In the study by Kent, et al.,[9] serum C4 was measured in 17 dogs with chronic diarrhea and 20 control dogs. Eleven of these cases were diagnosed with IBD. While there was no significant difference in the serum C4 concentration between both groups, three dogs in the diseased group were above the cut-off value established in the 20 healthy dogs. One of the dogs was partially responsive to a trial with the bile acid sequestrant cholestyramine in combination with a dietary trial. The authors suggested that bile acid malabsorption may be a clinically relevant disorder in dogs with chronic diarrhea.

The treatment of choice in humans with BAD is administration of bile acid sequestrants. In a recent study, patients with diagnosed BAD were followed over time for several years.[6] Of these, 38% were using bile acid sequestrants long term, and their stool frequency decreased from seven stools per day to three stools per day. In contrast, those patients who ceased treatment with bile acid sequestrants had no change in their stool frequency.

A recent study has shown an increased serum D-lactate concentration in cats with various gastrointestinal diseases.[10] The D-enantiomer of lactic acid is not normally found in any appreciable quantities in serum from mammals. The increase in serum D-lactate in cats with gastrointestinal disease is possibly due to disarrangements of the intestinal microbiota and increased bacterial production of D-lactate. D-lactate has been shown to lead to neurological signs in some cats.[11]

The above selected examples show that altered microbiota lead to systemic metabolic consequences. However, more in-depth studies evaluating additional metabolites are needed. More recent studies have utilized an untargeted metabolomics approach and measured several hundred metabolites in serum or feces of dogs with GI disease. One study evaluated the serum metabolite profile of dogs with idiopathic IBD before and after treatment. Serum and fecal samples were collected from healthy dogs (n = 10) and from dogs with IBD (n = 12) before and after 21 days of standard medical therapy (e.g., elimination diet and administration of anti-inflammatory drugs). A total of 359 serum metabolites were profiled using a GC-TOF/MS platform.[12] Serum concentrations of 3-hydroxybutyrate, hexuronic acid, ribose, and gluconic acid lactone

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were significantly more abundant in dogs with IBD. These metabolites are associated with the pentose phosphate pathway and indicate the presence of oxidative stress in dogs with IBD. Of interest is that the altered metabolic profiles did not normalize after three weeks of therapy, suggesting that the intestinal inflammation persisted despite improvement in clinical severity indices of these dogs. Another recent study in our laboratory evaluated the fecal metabolome in dogs with IBD. A total of 797 metabolites were analyzed using various mass spectrometry platforms. Significant alterations in various metabolic pathways were noticed. Dogs with IBD had altered SCFA metabolism (decreased propionate), altered bile acid metabolism, altered glycolysis pathways, and altered tryptophan-indole pathways. Furthermore, markers of oxidative stress were also highly altered in fecal samples of dogs with IBD. Among the redox components acting in the GI lumen, cystine, cysteine-s-sulfate, and cysteine, were increased in disease. Although glutathione disulfide and glutathione were not detected, 5-oxoproline was increased along with several gamma-glutamyl amino acids. Changes in components of the gamma-glutamyl cycle may suggest sequestration of intracellular glutathione by enterocytes or luminal bacteria under oxidative stress driven by abundance of leukocytes and production of reactive oxygen species. Abnormalities in the redox equilibrium were further supported by increases in the cystine/cysteine redox pair.

Effects of Antibiotics and Fecal Microbiota Transplantation

Antibiotic administration, such as metronidazole or tylosin has proven to be useful in treatment of GI disease, such as in mild feline IBD, chronic colitis, or presumed small intestinal dysbiosis.[13,14] Enrofloxacin ameliorates clinical signs and improves histologic lesions in dogs with granulomatous colitis.[15] The effect of antibiotics is most likely a combination of decreases in total bacterial load, changes in specific bacterial groups, and direct immunomodulation. For example, metronidazole increases *Bifidobacterium* populations and reduces the colonic oxidative damage to proteins. Tylosin has been shown to alter microbiota, but also a direct anti-inflammatory effect of tylosin has been proposed.[16] This anti-inflammatory effect has been speculated to be due to the modulation of cyclooxygenase-2, nitric oxidase synthase, and several cytokines.[17]

Antibiotic usage, however, has a very pronounced effect on the intestinal microbiome and may disrupt the microbial ecosystem for prolonged periods of time. In one study evaluating the fecal microbiota of healthy humans, approximately 30% of all bacterial taxa were affected, some of them for up to six months.[18] Similarly, administration of tylosin for 14 days led to significant modifications in the jejunal microbiota of dogs, with some bacterial groups depressed for more than the study period of 14 days.[19] Recent controlled studies are appreciating that antibiotics cause severe disruptions in major metabolic pathways, including bile acid metabolism and increase oxidative stress.[20] Furthermore, these metabolic changes are associated with clinical signs of GI disease in a subset of animals. For example, 14-day administration of metronidazole to healthy pet dogs led to development of diarrhea in 9/16 dogs, while on metronidazole, which resolved after the end of administration.[21] Similarly, 13/18 dogs that received intramuscular injections of lincomycin developed diarrhea.[22] Also, in a recent study, 34 cats were receiving amoxicillin-clavulanate twice daily for seven days, and the majority of cats developed signs of GI disease with worsening of fecal scores.[23] New epidemiological data from human medicine suggests that antibiotic-induced dysbiosis in early childhood is clinically relevant, as it is one of the major risk factors for the development of chronic diseases such as allergies and IBD.[24] Therefore, antibiotics usage and its potential side effects require careful study in dogs and cats.

Fecal microbial transplantation (FMT) has been used successfully in humans with recurring *C. difficile* infections and has been shown to lead to normalization of the microbiota. Studies have shown that this procedure is generally safe and effective in approximately 90% of patients with recurring or therapy-resistant *C. difficile* infection, leading to resolution of clinical sign within one to two days of administration. FMT has also been used for IBD, IBS, idiopathic constipation, and other non-GI diseases in humans, but the success rate is drastically lower than in *C. difficile* infection. This is likely due to the differences in pathophysiology of these disorders. While *C. difficile* infection is a classical dysbiosis, the cause for IBD is more complex and multifactorial, and the dysbiosis is just one component of the disease process. However, anecdotal reports have recently emerged suggesting that FMT can lead to improvement in clinical signs and dysbiosis in a subset of patients with chronic enteropathies. Unfortunately, at this point there are no diagnostic tests available that would allow clear predictions as to which patients will respond to FMT. Furthermore, no validated protocols are available, and some authors infuse FMT via nasogastric tube, while others administer it as colonic enema. One study described the use of FMT in dogs with refractory diarrhea, administered as an enema in unsedated patients.[25] The protocol uses a dose between 0.5 to 5 grams of stool per kg BW of the patient, which is blended with 60 ccs saline on the highest setting. The mixture is then aspirated into a 60 cc catheter tip syringe and attached to a red rubber catheter that is inserted into the colon and FMT is given as enema. The FMT was repeated up to three times every three to four weeks in some patients. Another recent small study evaluated three dogs with chronic enteropathies, with two of those being tylosin-responsive.[26] All three dogs showed an immediate reduction in the dysbiosis index,[27] but after three weeks, the dysbiosis returned in one dog, and this dog also did not show improvement

in clinical signs. In the remaining two dogs, both having tylosin-responsive diarrhea, a partial improvement in clinical signs was observed. This initial data suggests a potential for the use of FMT in patients with chronic enteropathies, but larger studies are needed to elucidate the clinical use of FMT in small animal medicine.

Conclusions

There is mounting evidence that microbiota dysbiosis is linked with metabolic changes in dogs and cats with GI disease. Future comprehensive studies evaluating the microbiota and the metabolome, as well as the host response via transcriptome are needed to better understand what therapeutic approaches will have the most important impact on the host to counterbalance these metabolic changes.

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