別記様式第３号

学　位　論　文　要　旨

氏　　名　　SUGITA, Koji

題　　目　Study on Clinical Efficacy and Mechanisms of Fecal Microbiota Transplantation in Dogs
（犬における糞便移植療法の有効性とメカニズムに関する研究）

Intestinal dysbiosis is characterized by reduction and disruption of the gut microbiota diversity. The presence of intestinal dysbiosis has been shown to be involved in the development of a variety of chronic diseases such as insulin-resistant type 2 diabetes, metabolic syndrome, inflammatory bowel disease (IBD), allergic diseases, and depression in humans. In dogs, intestinal dysbiosis was reported in IBD and myxomatous mitral valve disease. However, it is currently unclear whether intestinal dysbiosis is a cause or a consequence of these diseases in dogs.

Fecal microbiota transplantation (FMT) is a treatment performed by introducing fecal microbiota obtained from a healthy individual into the gastrointestinal (GI) tract of a diseased individual. The mechanisms underlying the effects of FMT are not fully understood but may be associated with enhanced numbers of beneficial microbes, increased microbial diversity, and restoration of normal microbiota. FMT has already become an effective treatment option for recurrent *Clostridium difficile* infection (CDI) in humans. FMT was also shown to be effective for non-GI diseases such as autoimmune diseases, metabolic diseases, and depression in humans. Published papers have demonstrated that FMT was effective for the treatment of several GI diseases in dogs, including canine parvovirus infection, post-weaning diarrhea, acute diarrhea, and IBD. However, since clinical data of FMT are limited in dogs, it remained unclear what diseases can be a target for FMT. Therefore, the purpose of this thesis was to investigate clinical efficacy, safety, and mechanisms of FMT in dogs with GI and non-GI diseases.

In chapter 1, I established a method of oral FMT and evaluated the therapeutic efficacy of a single oral FMT for canine *C. difficile*-associated diarrhea. Fresh feces collected from a healthy donor dog were dissolved in tap water, and then filtered through a medical gauze pad twice. The fecal solution was orally administered to a recipient dog with *C. difficile* associated diarrhea using a syringe. Stool consistency and frequency and fecal blood and mucus became normal 2–3 days after a single oral FMT, and real-time PCR analysis and immunochromatography was negative for *C. difficile* antigen and toxin A&B genes and proteins. No adverse events were observed. These results suggest that the cause of *C. difficile*-associated diarrhea in the dog may have been mainly intestinal dysbiosis due to toxins produced by *C. difficile*. It was highly likely that the transfer of a large number of beneficial bacteria by oral FMT normalized dysbiosis in the dog.

In chapter 2, I investigated the clinical efficacy of endoscopic FMT and changes in fecal microbiota in a dog with non-responsive enteropathy (NRE), a type of IBD. The dog with NRE showed intestinal dysbiosis. The fecal microbiota of the dog was predominantly composed of three phyla,

including Firmicutes, Bacteroidota, and Proteobacteria, whereas that of the donor dog was composed of five phyla, including Firmicutes, Fusobacteriota, Bacteroidota, Proteobacteria, and Actinobacteriota. Fresh feces obtained from a healthy donor were dissolved in saline, and then filtered through a medical gauze pad twice. The cecum and colon were thoroughly washed with saline just before FMT. The fecal solution was transplanted into the cecum and colon of the dog with NRE using endoscopy. As a result, clinical signs and dysbiosis were dramatically improved, and recurrence was not observed after FMT. No adverse effects were observed after endoscopic FMT. The dog with NRE newly acquired Fusobacteriota and Actinobacteriota in the gut microbiota. Dogs with NRE are poorly responsive to standard treatment and have a poor prognosis. The findings in chapter 2 suggest that FMT may be a promising treatment option for NRE.

In chapter 3, I analyzed the fecal microbiota of dogs with atopic dermatitis (AD) and evaluated the effects of a single oral FMT on clinical signs and fecal microbiota in dogs with AD as a non-GI disease. Dogs with AD was found to have intestinal dysbiosis compared to age-, breed-, and sex-matched healthy dogs. The fecal microbiota of dogs with AD was predominantly composed of three phyla, including Firmicutes, Bacteroidota, and Proteobacteria, whereas that of HC dogs and donor dogs was composed of five phyla, including Firmicutes, Fusobacteriota, Bacteroidota, Proteobacteria, and Actinobacteriota. The alpha diversity (richness and Shannon index) of the fecal microbiota was significantly lower in dogs with AD than in HC dogs. Unweighted and weighted UniFrac distances of the fecal microbiota were significantly different between dogs with AD and HC dogs. These findings indicated the presence of intestinal dysbiosis in dogs with AD. Therefore, I next performed a single oral FMT for dogs with AD. A single oral FMT improved the clinical signs by correcting the gut microbiota of dogs with AD. The dogs with AD newly acquired Fusobacteriota and Actinobacteriota in the gut microbiota. Furthermore, the positive correlations between the number of acquired amplicon sequence variants and improvement ratios of clinical scores were detected. Although four of 12 dogs with AD showed mild soft feces for a short-term after oral FMT, the causal relationship was unclear. The findings in chapter 3 suggest that FMT may be effective treatment option for cAD.

Currently, there is no consensus regarding the FMT methods in dogs. In this thesis, I selected oral FMT for a dog with *C. difficile*-associated diarrhea and dogs with AD and endoscopic FMT for a dog with NRE. Both methods improved clinical signs by correcting intestinal dysbiosis. These findings suggest that the FMT methods should be modified according to target diseases.

In this thesis, dogs with NRE and AD showed intestinal dysbiosis. Notably, Fusobacteriota was rarely detected in the feces of dogs with NRE and AD before FMT. Fusobacteriota and Actinobacteriota appeared in the feces of the recipient dogs after FMT, suggesting that these phyla may be crucial in the normal gut microbiota of dogs and involved in the mechanism of action of FMT. Further studies are needed to verify the function of these bacteria in dogs.

In conclusion, this thesis demonstrated that FMT could be an effective and safe treatment option for canine GI and non-GI diseases in which intestinal dysbiosis plays a crucial role in the pathogenesis. To expand application of FMT for other canine diseases, further evaluation of FMT, including mechanisms, safety, and modification of the method for improved clinical outcomes, should be performed.

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