論文審査及び最終試験又は学力の確認の結果の要旨

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学位論文名	Dietary Fiber Deficiency Accelerates Colitis in Mice in the Short Term Independent of Short-Chain Fatty Acids	
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論文審査の結果の要旨

食物繊維の摂取は潰瘍性大腸炎の症状改善や寛解維持に有効であるとされており、特に食物繊維の腸内細菌による代謝産物であるShort chain fatty acids (SCFAs) は腸内の各種細胞の生存維持や腸粘膜バリア機能維持に寄与する可能性が示唆されている。しかしながら一方で食物繊維の効果については懐疑的な報告もある。そこで本研究では大腸炎に対する食物繊維の防御的な効果を検証するため、マウスを用いて食物繊維の完全欠損状態が大腸炎を悪化させるか否かを検討した。大腸炎誘発試薬であるDextran sulfate sodium (DSS) 2% をマウスに5日間投与することで軽度な大腸炎を発症するモデルを用いて検討を行った。DSS の投与と同時に食物繊維完全欠損食 (Fiber Free diet, FF) または通常食 (Normal Diet, ND) を給餌し、各種指標にて大腸炎の程度を評価した。FF/DSS群では処置の1日後から下痢が生じ、経時的に体重の減少と血便の悪化が観察された。さらに処置1日から2日後には大腸の短縮、粘膜上皮の障害、好中球の指標であるMyeloperoxidase (MPO) 活性の増加、糞便中の SCFAs の減少が認められた。これらの変化は ND/DSS群においては非常に軽微、あるいは殆ど認められなかった。そこでSCFAsを投与して大腸炎の悪化が改善するか否かを検討したが、改善効果は認められなかった。一方でFF処置により1日後から多くの腸内細菌の存在比率が大きく変化するDysbiosis状態が認められた。特に腸炎悪化に関わる可能性が高いとされている*Desulfovibrio*属の増加と、大腸炎に対して抑制的に働くとされている *Lactobacillus*属の減少が認められた。これらの結果から食物繊維の欠損は短期間に腸内細菌叢の劇的変化であるDysbiosisを引き起こし、その結果、大腸炎を悪化させる可能性が考えられた。これらのことから食物繊維の継続的な摂取が潰瘍性大腸の予防や腸内環境の維持に重要であることが示唆された。

最終試験又は学力の確認の結果の要旨

申請者は腸炎モデルマウスを用いて、食物繊維の欠損が腸内細菌のバランス異常を引き起こす事によって腸炎を悪化させる可能性を示し、食物繊維の継続的摂取が腸炎の悪化防止に重要である事を示唆した。発表は明快で周辺知識も豊富であり、質疑応答も的確である事から、博士の学位授与に値すると判断した。　（主査：　岸　博子）

申請者は実験動物を用いて大腸炎に対する食物繊維の有用性を調べ、食物繊維の欠損がDysbiosisを引き起こし、大腸炎を悪化させる可能性を示唆し、食物繊維の継続的摂取が重要であり得ることを導いた。関連領域の知識も豊富であり、博士の学位授与に値する。　（副査：　竹下　治男）

申請者は、腸炎モデルを用いて、食物繊維が腸炎の病態に影響を与えるかを検討し、食物繊維の欠損が、短期間に腸内細菌のdysbiosisを引き起こし腸炎の悪化に関与する可能性を示した。この結果は大腸炎の病態解明につながる重要な成果である。発表は明快で質疑応答も的確であったことから、学位授与に値すると判断した。　（副査：　石村　典久）

（備考）要旨は、それぞれ４００字程度とする。

学 位 論 文 の 要 旨

氏名　神田翔磨

学 位 論 文 名　Dietary Fiber Deficiency Accelerates Colitis in Mice in the Short Term Independent of Short-Chain Fatty Acids

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論 文 内 容 の 要 旨

INTRODUCTION

Dietary fiber is considered to keep microbiota healthy state, that may contribute the intestinal barrier function and may increase resistance to colitis-inducing stimuli. In addition, short-chain fatty acids (SCFAs), the bacterial metabolites of dietary fiber, provide intestinal epithelial cells with energy and contribute to maintain epithelial barrier function. Based on these protective property of dietary fiber, intake of dietary fiber is recommended to keep remission of ulcerative colitis. However, protective effects of dietary fiber on colitis are controversial in animals and human. In our present study, we examined whether colitis is caused or worsened under fiber-deficient condition in mice to verify the protective effects of dietary fiber on colitis. In addition, we also examined the protective effect of SCFAs on aggravation of colitis due to fiber deficiency. Furthermore, we evaluated the changes in microbiota due to fiber deficiency to evaluate dysbiosis, that could cause vulnerability in barrier function.

MATERIALS AND METHODS

Mice were administered 2% dextran sulfate sodium (DSS), a colitis inducer, through drinking water. Along with the DSS-administration, mice were fed normal diet or fiber deficient diet. The impact of fiber deficient diet alone without DSS was also examined. Therefore, we made four groups: normal diet without DSS (ND group); fiber-deficient diet without DSS (FF

group); normal diet with DSS (NDD group); fiber deficient diet with DSS (FFD group). To evaluate the severity of colitis, diarrhea, blood in stool, and weight loss were evaluated as macroscopic changes that are associated with colitis. In addition, myeloperoxidase (MPO) activity and histopathological changes in the large intestine were assessed as direct indicators of colitis. To evaluate the involvement of SCFAs in the aggravation of colitis, cecum weight and the amount of SCFAs in the cecum, where SCFAs are produced, were measured. In addition, mixture of SCFAs (the mixture of acetate, propionate and butyrate) were administered orally during the intervention of fiber-deficient diet and DSS, then the colitis-associated parameters were evaluated. Furthermore, the percentages of major bacteria, which comprise more than 1% of the microbiota, were calculated to evaluate the dysbiosis. All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University.

RESULTS AND DISCUSSION

The diet feeding and DSS administration were carried out for 5 days (day 0 to day 5). The episodes of diarrhea occurred from day 1 and continued thereafter in the FFD group. There was no diarrhea in NDD group. Blood stool emerged from day 1 and got severer thereafter in the FFD group whereas no or mild blood stool was observed in NDD group. In addition, marked histopathological changes including disruption of epithelium and ulcers were observed in FFD group. In contrast, damage to epithelium and entire villi were mild in NDD group. These abnormal stool state and histopathological changes were not observed both in the ND group and FF group. Furthermore, FFD group showed higher MPO activity compared to NDD group although the MPO activities of both groups were higher than those of ND group and FF group. The MPO activity was not increased in FF group compared to ND group. These results indicate that a fiber deficiency accelerates DSS-induced colitis.

Given the early onset of diarrhea and blood stool in the FFD group, we evaluated the colitis-associated symptoms and direct intestinal damage at day 1 and day 2 to estimate the onset of the aggravation of the colitis. In the comparison of NDD group and FFD group, FFD group showed that enhancement of epithelium damage and MPO activity occurred from day 1 and day 2 respectively. These results suggest that fiber deficiency induced the vulnerability to colitis-inducing stimuli in a very short term.

The amounts of SCFAs in FF group and FFD group were smaller than those in ND group and NDD group. In addition, the amounts of SCFAs between FF group and FFD group, and ND group and NDD group were comparable respectively. The same tendency was observed in the cecum weight. These results indicate that fiber deficiency reduces the amount of SCFAs independent of colitis-inducing stimuli. Nevertheless, the administration of SCFAs did not ameliorate the worsening of the colitis. Therefore, the aggravation of the colitis due to fiber

deficiency would be independent of the decreased amounts of intestinal SCFAs.

In the comparison of the microbiota between the ND group and the FF group, the populations of almost all bacteria were different. The similar tendencies were observed between NDD group and FFD group. These changes were observed day 1 and continued thereafter. Among them, the increased population of *Desulfovibrio* spp. in FF group and FFD group was noteworthy since *Desulfovibrio* spp. are sulfate-reducing bacteria and produce hydrogen sulfide, which likely induce the destruction of epithelial cells as well as upregulation of proteolysis-inducing genes in colonocytes at higher concentration. In addition, the vesicles of the outer membrane destroy the epithelial barrier. Furthermore, *Desulfovibrio* spp. is abundant in IBD patients and positively correlates with the disease activity. Conversely, there have been multiple reports indicating a decrease in the population of *Desulfovibrio* spp. alongside the resolution of colitis in animal models. Therefore, increased *Desulfovibrio* spp. is potential causative factor, which induces vulnerability to DSS-stimulation. Besides, the populations of *Lactobacillus* spp. were lower in FF group and FFD group. Since multiple *Lactibacillus* species have been found to ameliorate DSS-induced colitis, decreased *Lactibacillus* spp. and increase in *Desulfovibrio* spp. instead may have accelerated the formation of unhealthy microbiota that makes intestinal tract irritable to colitis-inducing stimuli.

CONCLUSION

Deficiency of dietary fiber aggravates DSS-induced colitis independent of SCFAs in the short term and the dysbiosis is potential causative factor. These results suggest that a regular intake of dietary fiber is strongly recommended to avoid colitis and preserve intestinal health.