学位論文内容の要旨

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学位論文題名

Induction of ileal permeability and alterations in metabolome in the gut–liver axis induced by 12α-hydroxylated bile acids in rats

（ラットにおける12α水酸化胆汁酸による回腸透過性の誘導と腸肝軸における代謝物の変動）

A high-fat diet is associated with and may contribute to the prevalence of a series of gut–liver diseases and metabolic disorders. Bile acids (BAs) are cholesterol catabolites. Both the level and composition of BAs are regulated by the amount of dietary fat. 12α-hydroxylated (12αOH) BAs are selectively increased under the condition of a high-fat diet and may influence hepatic steatosis and gut barrier dysfunction, but the mechanism remains unclear. High-fat diet raises the fecal output of deoxycholic acid (DCA), which disrupts epithelial integrity. An increase in fecal DCA level implies the accumulation of primary 12αOH BAs in the enterohepatic circulation. However, the potential role of abundant primary 12αOH BAs in gut barrier dysfunction has not been studied yet. In addition, dietary supplementation of cholic acid (CA) at a level that does not disturb 7α-dehydroxylation in the large intestine induces hepatic steatosis without obesity in rats. The underlying mechanism by which 12αOH BAs induce gut barrier dysfunction and hepatic steatosis has not been fully clarified. The purposes of this study were to investigate the role of primary 12αOH BAs in gut barrier impairment and effect of 12αOH BAs on metabolic alterations in hepatic steatosis induced by the dietary CA supplementation.

To examine the role of primary 12αOH BAs in gut barrier impairment, rats were fed a CA-supplemented diet (0.5 g/kg diet). The CA diet increased the 12αOH BA concentrations in the small and large intestine, accompanied by gut barrier impairment. Based on the luminal 12αOH BA concentrations, *ex vivo* gut leakiness was determined. DCA increased permeability in the large intestine, whereas taurocholic acid (TCA) increased the ileal permeability, but not jejunal permeability. A Rho kinase inhibitor attenuated TCA-induced ileal permeability. Administration of vancomycin that abolishes secondary BA production did not influence the gut leakiness induced by the

CA diet. Changes in the gut permeation marker in the tail vein blood suggested that the major site of the CA-induced leakiness was small intestine. The CA diet enhanced the phosphorylation of myosin light chain 2 and reduced claudins expressions in the rat ileal epithelia. Such alteration of the expression of barrier function-related genes was not clearly observed in the large intestine. These observations suggest a primary event in 12αOH BA-induced gut leakiness is the TCA-induced ileal barrier dysfunction.

Next, to investigate the gut–liver metabolic responses to 12αOH BAs, rats were fed the CA-supplemented diet. After 12-weeks of the dietary CA intervention, the hepatic lipid accumulation was greater in the CA-fed rats than in control without increase in dietary energy absorption. Untargeted metabolomics suggested marked differences in the fecal metabolome between the CA-fed rats and control, which was characterized by depletion of fatty acids and enrichment of amino acids and amines. Moreover, an alteration of molecules in redox-related pathways was observed in liver metabolomics in the CA-fed rats. The CA diet tended to reduce hepatic concentration of nicotinamide adenine dinucleotide and enhanced poly(ADP-ribosyl)ation of peroxisome proliferator-activated receptor α (PPARα), which suggests that an activation of poly(ADP-ribose) polymerase-1 impaired PPARα signaling in the liver of the CA-fed rats. The ingestion of the CA diet increased the sedoheptulose-7-phosphate level and enhanced glucose-6-phosphate dehydrogenase activity in the liver, suggesting promotion of the pentose phosphate pathway that contributes to increase in reduced nicotinamide adenine dinucleotide phosphate in the liver. Integrated analysis of the gut–liver metabolomics revealed that DCA produced in the large intestine is the major mediator in these metabolic alterations.

Overall, this study demonstrated the significance of TCA in proximal gut leakiness, and alterations in metabolites induced by DCA in the gut–liver axis contributed to the enhancement of liver lipid accumulation. These results provide a novel perspective on how 12αOH BAs regulate the pathophysiology of the gut–liver axis.