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Evaluation of gut dysbiosis using serum and fecal bile acid profiles

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Abstract

Dysbiosis in the intestinal microflora can affect the gut production of microbial metabolites, and toxic substances can disrupt the barrier function of the intestinal wall, leading to the development of various diseases. Decreased levels of Clostridium subcluster XIVa (XIVa) are associated with the intestinal dysbiosis found in inflammatory bowel disease (IBD) and *Clostridium difficile* infection (CDI). Since XIVa is a bacterial group responsible for the conversion of primary bile acids (BAs) to secondary BAs, the proportion of intestinal XIVa can be predicted by determining the ratio of deoxycholic acid (DCA)/[DCA + cholic acid (CA)] in feces orserum. For example, serum DCA/(DCA+CA) was significantly lower in IBD patients than in healthy controls, even in the remission period. These results suggest that a low proportion of intestinal XIVa in IBD patients might be a precondition for IBD onset but not a consequence of intestinal inflammation. Another report showed that a reduced serum DCA/(DCA + CA) ratio could predict susceptibility to CDI. Thus, the BA profile, particularly the ratio of secondary to primary BAs, can serve as a surrogate marker of the intestinal dysbiosis caused by decreased XIVa.

INTRODUCTION

The human gut contains 10^{14} bacteria, ten times the number of human cells, which constitute 150 times more genes than the human genome[1]. Dysbiosis refers to an alteration of normal healthy state of the microbiota[1]. Alterations in the intestinal microbiota change the metabolites of gut microbiota, and toxic substances can disrupt intestinal barrier function and cause various diseases[2]. Dysbiosis is mainly associated with digestive disorders such as ulcerative colitis (UC)[3-6], Crohn's disease (CD)[3,4,7,8], irritable bowel syndrome (IBS)[9], non-alcoholic fatty liver disease[10,11], and hepatocellular carcinoma[12]. In addition to digestive disorders, diabetes[13], atherosclerosis[14,15], obesity[16], atopies and asthma[17], and multiple sclerosis[18] have been associated with dysbiosis.

Since the 1990s, molecular biological techniques for the detection of dysbiosis have advanced rapidly since the 1990s, and methods utilizing the bacterial 16S rRNA gene variable region have allowed investigation of the gut microbiota[19]. Furthermore, shotgun metagenomics approaches utilize untargeted sequencing methods to capture all microbial genomes[20]. However, all of these methods require collection of fecal samples, and measurement and data analysis are time-consuming.

Bile acids (BAs) are secreted from the liver into the bile. An active transport system takes up approximately 95% of biliary BAs at the end of the ileum[21], and the remaining 5% is carried to the colon while some are absorbed passively. The absorbed BAs return to the liver through the portal vein called enterohepatic circulation. Intestinal bacteria convert the structure of BAs in the gut, and the converted BAs are present in the feces and enterohepatic circulation. Because a certain quantity t of BAs in the enterohepatic circulation leaks into the peripheral blood, the BA profiles in the feces as well as the peripheral blood may serve as markers of gut microbiota composition.

Dysbiosis has been reported in several gastrointestinal diseases, especially a reduced *Clostridium* subcluster XIVa (XIVa). XIVa is a major bacterial group that metabolizes BAs in the human gut[22,23]. Therefore we have a new hypothesis that the fecal and serum BA profiles could be a useful biomarker for intestinal XIVa activity. We have demonstrated the new facts that fecal and serum ratios of DCA/(DCA+CA) are

useful as surrogate indicators of the gut proportion of XIVa, including the inflammatory bowel diseases (IBD) and *Clostridium difficile* infection (CDI)[24,25]. The unique insight of this review is that this review focused on the studies using the BA calculated product/(product+substrate) ratio, which is not discussed enough in previous reviews. We believe these results are useful in clinical practice, and it is necessary to investigate various diseases in the future studies.

In this review, we summarize the current literature regarding the relationship between BAs and the gut microbiota and the application of fecal and serum BA profiles as surrogate markers of dysbiosis and associated digestive disorders.

BA METABOLISM BY INTESTINAL MICROBIOTA

BAs are the end products of cholesterol metabolism. The human liver synthesizes glycine or taurine conjugated cholic acid (CA) and chenodeoxycholic acid (CDCA). These primary BAs are excreted into the bile and transported to the intestine. In the terminal ileum and the large intestine, the intact primary BAs are modified by intestinal bacteria (Figure 1). In this process, glycine and taurine are initially deconjugated by the bile salt hydrolases (BSH) expressed in various bacteria. Then, the hydroxyl group at the C-7α position of CA and CDCA is dehydroxylated, and the secondary BAs, deoxycholic acid (DCA) and lithocholic acid (LCA), are formed. This dehydroxylation step is catalyzed by a multi-step reaction encoded by bile acid-inducible (bai) genes in a single bai operon[21,26]. These bai genes are present only in specific bacteria, which account for nearly 0.0001% of the total colonic flora[21]. In addition, hydroxyl groups at the C-3α, 7α, and 12α positions of both conjugated and unconjugated BAs can be dehydrogenated to carbonyl groups and further epimerized to 3β-, 7β- and 12β-hydroxyl groups by intestinal bacteria[21].

The relationship between the enzymatic transformation of BAs and intestinal bacteria has been studied previously[21]. The deconjugation of amino acids is carried out by a variety of bacteria, including *Bacteroides*, *Peptostreptococcus*, *Clostridium*, *Streptococcus*, *Eubacterium*, *Lactobacillus*, and *Bifidobacterium*[27]. In contrast, multi-step 7α-

dehydroxylation of BAs is mediated by *Clostridium* cluster IV (*C. leptum*)[28], cluster XI (*C. sordellii*, *C. hiranonis*, and *C. bifermentans*)[29], and cluster XIVa (*C. scindens* and *C. hylemonae*)[30], of which cluster XIVa is reported to play a major ~~central~~ role in this transformation[21,31].

CLOSTRIDIUM SUBCLUSTER XIVA AND GASTROINTESTINAL DISEASES

The interaction between gut microbiota and BAs has been implicated in the pathogenesis of various disease states, including IBD, CDI, IBS, asthma, and obesity[32]. In these diseases, alterations of gut microbiota are associated with decreased BA deconjugation (or BSH activity) and/or reduced secondary BA production[32].

A reduced proportion of XIVa and decreased levels of secondary BAs have been reported in dysbiosis-associated gastrointestinal diseases, including IBD[22,23], CDI[33-36], and liver cirrhosis[31,37]. These results suggest that BA composition is markedly affected by the number of XIVa. Conversely, the number of XIVa is affected by intestinal CA amount[31]. Patients with liver cirrhosis have decreased intestinal XIVa and DCA levels due to the reduced size of the CA pool[31,37]. This is in contrast to the findings associated with a high-fat diet, which stimulates the biliary secretion of CA and increases intestinal XIVa and DCA levels[38,39].

Previous reports have also indicated that changes in the intestinal microbiota and increased DCA levels may lead to morbidity, including colon[40] and liver cancers[12]. Epidemiological evidence suggests that colorectal cancer is associated with increased levels of DCA in serum, bile, and stool[40]. Therefore, the benefits of increased XIVa and DCA in patients with gastrointestinal diseases are a topic of debate.

BAS AS BIOMARKERS FOR DYSBIOSIS DETECTION

As mentioned above, deconjugation of BAs is easily mediated by the major bacteria, and most of the conjugated BAs in serum reflect the BAs reabsorbed without exposure to these bacteria. Therefore, the unconjugated form of BA is a better marker than total (conjugated + unconjugated) BA to calculate the BA-transformation activity of intestinal

microbiota. For estimation of the approximate activity, the product/(product+substrate) ratio was calculated in a previous study[24]. In this approach, 7α-dehydroxylation was estimated by calculating DCA/(DCA+CA) or LCA/(LCA+CDCA). Epimerization of 3αOH BAs to 3βOH BAs is divided into two reactions. The conversion of 3αOH BAs to 3oxo BAs was estimated by 3oxo BAs/(3oxo BAs+3αOH BAs), and that of 3oxo BAs to 3βOH BAs was estimated by 3βOH BAs/(3βOH BAs+3oxo BAs). Epimerization of 7αOH BAs to 7βOH BAs and 12αOH BAs to 12βOH BAs was also estimated in the same way. The highest correlations were obtained between the proportion of fecal XIVa and fecal DCA/(DCA+CA) and serum DCA/(DCA+CA) (Table 1). In addition to DCA/(DCA+CA), LCA/(LCA+CDCA) is another marker for 7α-dehydroxylation, but its correlation coefficient with XIVa was lower than that of DCA/(DCA+CA). In healthy subjects, the ratios of the LCA/(LCA+CDCA) are much smaller than those of DCA/(DCA+CA) in serum but not in feces[24], suggesting that LCA is not easily absorbed from the intestine than other BAs. Therefore, as a serum marker for 7α-dehydroxylation, DCA/(DCA+CA) is better than LCA/(LCA+CDCA). Thus, by measuring the DCA/(DCA+CA) ratio in feces or serum, the abundance of XIVa and presumably the presence of dysbiosis can be estimated[24]. As shown in Table 2, these product/(product+substrate) ratios of BAs are now being applied in several studies, including those involving IBD patients[24] and CDI patients[25], studies on the effects of a high-fat diet in mice[41], and studies on the effects of water-soluble dietary fiber in humans[42]. Furthermore, the product/(product+substrate) ratios of fecal BAs can be calculated from the fecal BA data shown in the previous studies[43,44].

BA METABOLISM IN PATIENTS WITH IBD

IBD is a chronic inflammatory condition of the colon and small intestine. The two forms of IBD, UC and CD, overlap clinically and pathologically, but are often very different[45,46]. The etiology of IBD remains unknown, but is believed to be attributable to the interaction of genetic and environmental factors.

The relationship between BAs and IBD has been reported in multiple studies[47-60]. The interaction of BAs and gut microbiota has been suggested to be closely related to the pathogenesis of IBD[48]. The fecal dysmetabolism of BAs observed in IBD is linked to IBD-associated dysbiosis, indicating that BA dysmetabolism could be used as a surrogate marker of IBD[51]. To evaluate the role of BAs in intestinal inflammation, the metabolomic, microbiome, metagenomic, and transcriptomic profiles of stool from the ileal pouches in patients with UC were investigated and revealed that dysbiosis induced secondary BA deficiency, which promotes intestinal inflammation[52].

Bamba *et al*[61] recently investigated the relationship between the gut microbiota and BA composition in the ileal mucosa of CD. In their study, the proportion of conjugated BAs was significantly higher in CD patients than in controls and was positively correlated with the presence of genera such as *Escherichia* and *Lactobacillus* and negatively correlated with the presence of genera such as *Roseburia*, *Intestinibacter*, and *Faecalibacterium*. These results suggested that ileal mucosa-associated dysbiosis and the alteration of BA compositions of fluid in the ileum may influence the pathology of ileal lesions of CD[61].

Previous studies have confirmed that intestinal XIVa, as well as cluster IV, are significantly decreased in patients with CD[7,8] and UC[5,6]. Serum DCA/(DCA+CA) was examined in controls and IBD patients in remission and exacerbation periods, and was significantly lower in IBD patients than in healthy controls, even in the remission periods. These results show that the low proportion of intestinal XIVa proportion in IBD patients is not a consequence of intestinal inflammation but a precursor to the development of IBD[24].

Bile acid malabsorption (BAM) is one of the hallmarks of CD, and BAs are potential activators of PXR. Therefore, the relationship between BAM and PXR activity in CD patients was investigated. Serum concentrations of 7α-hydroxy-4-cholesten-3-one (C4), a marker for hepatic bile acid biosynthesis[62], and FGF19, a marker for intestinal BA flux[63], were compared among patients with CD and UC and control participants. C4 Levels in CD patients were significantly higher than those in controls. In particular, the

C4 values of CD patients with a history of ileal resection were markedly elevated and significantly higher than those of CD patients without a history of surgery. In contrast, serum FGF19 Levels in CD patients were significantly lower than those in UC patients, and tended to be lower than those in control individuals. CD patients with a history of ileal resection showed a marked decrease in the serum FGF19 concentration, which was significantly lower than those in CD patients without a history of surgery[47]. In addition, a significant negative correlation between 4β-hydroxycholesterol, a known marker for CYP3A4 activity, and C4 concentration was observed in CD patients. Since CYP3A4 is a target gene of PXR, the degree of BAM in CD patients was closely related to the deactivation of PXR. Thus, BA is a critical factor for the preservation of baseline activity of hepato-intestinal PXR in CD patients[47].

THE RELATIONSHIP BETWEEN BAS AND CDI

CDI is a common infection associated with hospitals and antibiotics. It causes a variety of clinical manifestations of colitis in healthcare facilities and the community[64-67]. Because CDI can be life-threatening, especially in the elderly, methods for screening high-risk hospitalized patients and preventing and treating CDI are desirable. Many reports have described the relationship between BAs and CDI. The secondary BAs, DCA and LCA, are more hydrophobic than the original primary BAs and have strong antimicrobial effects due to their high affinity with the lipids of cell membranes[68]. In addition to the bactericidal action, secondary BAs inhibits the proliferation of CD, the pathogen causing intractable diarrhea[33]. Previous reports have shown that DCA and LCA inhibit CD growth *in vitro*[69,70] and *in vivo*[71-74], and the levels of these secondary BAs in stool are reduced in CDI patients[32,75].

Regarding the relationship between indigenous enterobacteria and CDI, *Clostridium scindens*, one of the BA 7α-dehydroxylating bacteria, is associated with resistance to CDI[33,34]. In addition, fecal samples from CDI patients more frequently show negative results for bile acid-inducible (bai) genes than samples from control subjects, indicating that bai gene-positive species are involved in resistance to CD colonization[35]. More

interestingly, the bile acid 7α-dehydroxylating bacteria, *C. scindens* and *C. sordellii*, secrete tryptophan-derived antibiotics and inhibit CD growth. These antibiotics inhibit cell division of CD, and the secondary BAs such as DCA and LCA, but not CA, enhance the inhibitory activity of these antibiotics[36].

Although many reports have shown the relationships between BAs and CDI, studies using BA composition as a predictive surrogate marker to CDI susceptibility are limited[25,75]. Allegretti *et al*[75] showed that the fecal DCA to glycoursodeoxycholate (GUDCA) ratio was the best predictor and a potential biomarker for the recurrence of CDI. However, GUDCA is not a substrate of DCA, and GUDCA concentration is influenced by a number of factors other than BA 7α-dehydroxylation activity, including glycine/taurine conjugation ratio, deconjugation activity, the conversion rate of CDCA to ursodeoxycholate (UDCA) by 7-epimerization, and the possibility of UDCA administration to patients with hepatobiliary diseases. On the other hand, we showed that the serum DCA/(DCA+CA) ratio at the time of admission (before the use of antibiotics and CDI onset), was significantly low in patients who developed CDI while in the hospital compared to those in patients who did not develop CDI or in healthy controls[25]. In this study, DCA/(DCA+CA) < 0.349 was the cut-off value for discriminating patients at high risk of CDI before treatment with antibiotics, and the sensitivity and specificity of this threshold were 91.67% and 66.10%, respectively (Figure 2). The use of antibiotics represents the greatest risk factor for the development of CDI. However, patients who develop CDI already have a gut microbiota with significantly reduced diversity prior to antibiotic therapy[76].

CONCLUSION

Gut dysbiosis, particularly decreased XIVa, correlates strongly with decreased conversion of primary BAs to secondary BAs. Therefore, the DCA/(DCA+CA) ratio in feces and serum is a valuable marker for detecting dysbiosis caused by decreased XIVa without genetic analysis of enterobacteria.

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