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Original article

Analysis of fecal microbiota, organic acids and plasma lipids in hepatic cancer patients with or without liver cirrhosis

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SUMMARY

Background & aims: Changes in the microbiota composition are able to affect nutrient absorption and energy metabolism, but there are few human studies. The aims were to analyze fecal constituents quantitatively and compare them with liver dysfunction in hepatic cancer patients and to evaluate the relationships among intestinal microbiota, fecal organic acids and plasma lipid composition.
Methods: Fecal samples collected from 46 hepatic cancer patients (with liver cirrhosis, chronic hepatitis or liver fibrosis and normal liver) were evaluated for fecal constituents. Blood organic acid, lipid and fatty acid concentrations were analyzed.
Results: Fecal microbiota and organic acids showed no significant differences among different liver dysfunction patients. In normal liver patients, fecal *Candida* was positively correlated with plasma phospholipid while *Bifidobacterium* was negatively correlated with plasma eicosapentaenoic acid and eicosapentaenoic acid/arachidonic acid ratio (all $p < 0.05$). In cirrhotic liver patients, positive correlations were noted for *Lactobacillus* and docosahexaenoic acid and *Candida* and eicosapentaenoic acid or eicosapentaenoic acid/arachidonic acid ratio (all $p < 0.01$). It was suggested that intestinal biota affected serum fatty acid metabolism and were modified by liver disorders.
Conclusions: Intestinal microbiota and organic acid concentrations in hepatic cancer patients had positive and/or negative correlations with serum lipid levels.

1. Introduction

Recent studies have focused on gut microbiota as environmental factors that contribute to obesity and metabolic syndrome in humans.[1] The microbiota affects lipid composition and metabolism in serum, liver, and peripheral organs indirectly through modulation of gut hormones, and directly microbial modification of gut-derived lipids with several mechanisms.[2] In recent years an increasing amount of literature has demonstrated that several diseases are related to alterations in the intestinal microbiota (known as dysbiosis), such as irritable bowel syndrome, inflammatory bowel disease, diabetes, atopic disease, cancer and obesity.[3] These studies have mainly shown that differences in the composition of the intestinal microbiota are associated with disease. Animal studies have revealed how qualitative and/or quantitative changes in the microbiota composition are able to affect nutrient absorption and energy metabolism, but there are still a few human studies.[4]

Intestinal breakdown of indigestible carbohydrates and proteins, including prebiotics, leads to the production of substantial amounts of short-chain fatty acids (SCFAs), mostly acetate, propionate and butyrate, which are almost completely absorbed along the digestive tract.[4] The ratios of the SCFA concentrations in the colonic lumen are about 60% acetate, 25% propionate and 15%

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butyrate. While butyrate is widely metabolized by enterocytes, propionate and acetate reach the liver through the portal vein.[5] Recently, human studies of plasma SCFAs levels have indicated that intestinal SCFAs released from the gut are taken up by the normal and cirrhotic liver in humans.[6,7]

When portal acetate enters hepatocytes, it is mainly activated by cytosolic acetyl-coenzyme A synthetase 2, and enters the cholesterolgenesis and lipogenesis pathway.[5] Conversely, portal propionate, a substrate for gluconeogenesis, contributes toward decreases in cholesterolgenesis and lipogenesis in hepatocytes. The effect of acetate on reducing plasma free fatty acids levels has also been documented in humans.[8] The serum acetate/propionate ratio is related to the serum cholesterol level by the mechanism that colonic SCFAs influence systemic lipid metabolism.[9]

Recently, two orphan G-protein-coupled receptors, GPR41 and GPR43, were identified as receptors for SCFAs.[10,11] The potency order of the SCFAs for GPR41 is propionate > butyrate >> acetate, while GPR43 is equally sensitive to all three SCFAs. Acetate is more selective for GPR43 and GPR41. Karaki et al.[12] reported that GPR43 is expressed in enteroendocrine cells and mucosal mast cells in the rat intestine. Ge et al.[13] reported that activation of GPR43 by acetate causes a reduction in the plasma free fatty acid levels. These data suggest that the luminal microbiota and SCFA levels change the plasma fatty acid levels. However, detailed analyses of the relationships between intestinal SCFA concentrations and plasma lipid levels have not been reported.

The liver plays a central role in lipid metabolism, and various abnormalities in lipid metabolism have been reported in liver diseases, including the plasma phospholipid fatty acid pattern.[14] Damages in liver can result in the reduced blood flow going through the gut–liver axis, altered bile secretion and impaired peristalsis, which lead to the disruption of the mucosal barrier and the ecological balance of the gut microbiota. Altered gut biota in turn further contributes to complications associated with severe liver damage.[15] Regarding analyses of intestinal biota in cirrhotic patients, intestinal microbiota in patients with liver cirrhosis and the effects of probiotics on microbiota were reported by Zhao et al.,[16] who indicated that the imbalance of intestinal biota was matched by the severity of liver dysfunction. A recent report of gene-analysis of stool samples in cirrhotic patients also indicated increase of *Enterobacteriacae* and *Enterococcus*.[17] Tarao et al.[18] reported that lactitol, a non-absorbable synthetic disaccharide, increases *Bifidobacterium* and *Lactobacillus* with improvement of hepatic encephalopathy. And we reported that perioperative synbiotic treatment consisting of *Bifidobacterium*, *Lactobacillus*, and galactooligosaccharides attenuates the decrease in intestinal integrity and reduces infectious complications in hepatic surgery patients with or without liver cirrhosis.[19] However, very little is known about the relationships among intestinal microbiota and organic acid constituents and the resulting plasma lipid composition in normal healthy subjects and patients with liver cirrhosis.

We hypothesized that intestinal microbiota and organic acid concentrations in hepatic cancer patients have correlations with SCFAs and lipid metabolism. In this study, the relationships among intestinal microbiota, fecal organic acids, plasma lipid composition including polyunsaturated fatty acid (PUFA) and body mass index (BMI) were evaluated in hepatic cancer patients with or without pathologically diagnosed liver cirrhosis.

2. Materials and methods

2.1. Patients

This study involved 46 patients who were scheduled to undergo liver resection for a primary or metastatic liver tumor at the Hyogo Cancer Center between February 2005 and March 2008. Written informed consent for participation was obtained from each patient before enrollment in the study, which was approved by the Human Research Review Committee of Hyogo Cancer Center (Institute Identifier No. 131). All the patients received a regular diet preoperatively, and no patients received preoperative parenteral or enteral nutritional supplementation. There were 89 patients scheduled to undergo liver resection in this time period and 46 patients who accepted enrollment in this study and had sufficient fecal and plasma samples were selected for the analysis.

2.2. Study design and setting

The histological findings for the non-cancerous portion of the resected hepatic cancer specimens were divided into three groups: NL: normal liver; CH/LF: chronic hepatitis or liver fibrosis; and LC: liver cirrhosis. Fecal samples collected from the 46 hepatic cancer patients at the point of admission were evaluated for nine intestinal microbiota species and eight organic acid concentrations. Blood organic acid, lipid and fatty acid concentrations were analyzed. The BMI was calculated as the body weight (kg) divided by the square of height (m).

The study was designed to explore serum organic acid levels, fecal microbiota, fecal organic acid levels, routine blood cell counts and blood biochemical tests in the three groups. The correlations among fecal microbiota, fecal and serum organic acid concentrations, serum fatty acid concentrations, marker of liver injury in blood biochemical tests and BMI were compared among the three groups.

2.3. Fecal bacteriological examination

In this study, it was impossible to collect the human intestinal contents, and feces were used as test samples. One gram of fecal sample was added to 3 mL of sterilized anaerobic transfer medium (0.0225% (w/v) KH_2PO_4, K_2HPO_4 and $(NH_4)_2SO_4$; 0.045% (w/v) NaCl; 0.00225% (w/v) $CaCl_2$ and $MgSO_4$; 0.3% (w/v) Na_2CO_3; 0.05% (w/v) L-cysteine hydrochloride; 0.0001% (w/v) resazurin; 1.0% (w/v) Lab lemco powder (Oxoid Co., Ltd., Basingtoke, UK); 10% (w/v) Glycerol (Wako Chemical Co., Osaka, Japan))[20] in an atmosphere of 100% CO_2, immediately placed in cold storage, and sent to the Yakult Central Institute for Microbiological Research (Tokyo, Japan) for culture within 24 h. After serial dilution of the fecal suspensions with anaerobic buffer solution (0.0225% (w/v) KH_2PO_4, K_2HPO_4 and $(NH_4)_2SO_4$; 0.045% (w/v) NaCl; 0.00225% (w/v) $CaCl_2$ and $MgSO_4$; 0.3% (w/v) Na_2CO_3; 0.05% (w/v) L-cysteine hydrochloride; 0.0001% (w/v) resazurin), 50- or 500-μL portions of the diluents were spread onto the following culture media (roll tube agar: 500 μL, agar plate: 50 μL) under a 100% CO_2 gas jet with a gas jet system (SANSHIN INDUSTRIAL Co. Ltd. Kanagawa, Japan). In the gas jet system, 100% CO_2 gas, in which oxygen could be completely removed by passing CO_2 gas through a copper wire reduced with H_2 gas, was used. The diluted fecal suspensions were stored on ice in a refrigerator until culture. Diluted suspension was used to inoculate roll tube agar medium using a gas jet system, and agar plate medium in an anaerobic glove box which contained 88% nitrogen, 7% hydrogen, and 5% carbon dioxide. The test tube was refrigerated until culture. VL-G roll tube agar[21] supplemented with 0.2% cellobiose and 0.2% maltose (modified VL-G roll tube agar) was used to determine the total anaerobe counts. Various media were used for selective isolation of different microorganisms[21]: modified VL-G roll tube agar with addition of 80 μg/mL vancomycin (Sigma Chemical Co., St. Louis, MO) and 1 μg/mL kanamycin (Sigma) and heart infusion agar, supplemented with 0.2 μg/mL neomycin (Sigma), 0.01% (w/v) brilliant green, 0.1% (w/v) sodium taurocholate, 0.03% (w/v)