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Case report

Gut microbiota and environment in patients with major burns – A preliminary report

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ABSTRACT

Introduction: The gut is an important target organ after severe insult. Gut microbiota have an important role in immune response. However, the gut microbiota and environment have not been clarified in patients with burns. Therefore, we serially evaluated the gut microbiota and environment in patients with major burns.

Methods: Fecal samples from five patients with major burns were measured for quantitative evaluation of the gut microbiota.

Results: In the four survivors of major burns, the numbers of beneficial bacteria, especially those of total obligate anaerobes and *Bifidobacterium*, initially decreased, but then increased as the condition of the survivors improved. By contrast, the numbers severely decreased in the non-survivor as gut failure and sepsis progressed. The number of pathogenic bacteria such as *Pseudomonas* and *Candida* did not continue to increase in the survivors, whereas in the non-survivor the number increased and continued to higher counts. Short-chain fatty acids such as propionic and butyric acids decreased to lower-than-normal levels but tended to increase after recovery in the survivors. The levels remained below normal in the non-survivor.

Conclusions: The gut microbiota and environment are severely altered in patients with major burns. Consequently, abnormal gut conditions may have an influence on the systemic inflammatory response and multiple organ dysfunction syndrome. A novel treatment to maintain the gut microbiota and environment is expected in the future.

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1. Introduction

A systemic inflammatory response can continue over a long period after an extensive burn and can advance to systemic inflammatory response syndrome (SIRS), sepsis, and multiple organ failure. Studies have focused on the intestine as an important target organ during severe stress, and the importance of bacterial translocation has been pointed out as a cause for bacteremia. Animal studies have shown the ease of initiating bacterial translocation in conditions of apoptosis, increased permeability, and deteriorated gut microbiota in the intestine [1]. Moreover, it is reported that the collapse of intestinal barrier function causes inflammatory mediators to flow into the systemic circulation through mesenteric lymph channels and to become a cause of the progression of multiple organ dysfunction syndrome (MODS). Although there are many reports about the gut microbiota in animal studies, there are few clinical reports.

There are $>10^{11}$colony-forming units (CFU)/g of bacteria in adult feces. The intestinal bacteria have been reported to send host various signals related to the maintenance of intestinal barrier function and prevention of infection [2]. Shimizu et al. reported that the numbers of total obligate anaerobes in feces, which constitute the main population, are decreased in SIRS and are related to bacteremia and mortality in the intensive care unit (ICU) [3,4]. In this report, we show the serial changes of the gut microbiota and short-chain fatty acids (SCFAs) in patients with extensive burns.

2. Materials and methods

2.1. Patients

The subjects included five patients. Four patients survived (burn index 41.0 ± 20.0, age 41.5 ± 15.7 years), and one patient did not (burn index 60, age 37 years) (Table 1). All patients except Case 2 had inhalation injuries. All of the survivors could tolerate enteral nutrition and developed no gastrointestinal complications during their stay in the ICU. Septic complications of pneumonia occurred in two patients and bacteremia in one patient in the survivors, but they recovered. Both pneumonia and bacteremia occurred in the non-survivor. The clinical course of the non-survivor is described from the perspective of the gut microbiota and environment and dysmotility.

2.2. Methods

2.2.1. Bacteriological analysis

We analyzed the bacteriologic culture in each fecal sample that we collected at the same time as the collection for the SCFA measurement. For analysis of the microbiota, we also used 1g of feces. As mentioned above, the feces collected were maintained under anaerobic conditions with CO_2 saturation. The test tube was cooled in an icebox before culture. VL-G roll tube agar 9 supplemented with 0.2% cellobiose and 0.2% maltose (modified VL-G roll tube agar) was used to determine the total anaerobe counts. Different media were used for selective isolation of different microorganisms: modified VL-G roll tube agar to which 80 μg/ml vancomycin and 1 μg/ml kanamycin were added for Bacteroidaceae, *Clostridium welchii* (CW) agar (Nikken Bio Medical Laboratory Inc., Kyoto, Japan) for lecithinase-positive *Clostridium*; MPN agar[10] for *Bifidobacterium*; colistinoxolinic blood (COBA) agar[11] for *Enterococcus*; Lactobacillus selection (LBS) agar (Becton Dickinson and Company, Cockeysville, MD, USA) supplemented with 0.8% LabLemco powder (Oxoid Co., Ltd., Basingstoke, UK) for *Lactobacillus*; and *Staphylococcus* medium no. 110 agar for *Staphylococcus*, desoxycholate hydrogen sulfide lactose (DHL) agar for *Enterobacteriaceae*, nalidixic acid cetrimide (NAC) agar for *Pseudomonas*, and gelatin salt (GS) agar for *Candida* (all agars from Nissui Pharmaceutical Co., Ltd., Tokyo, Japan). The reproducibility and stability of these measurements were shown previously [5,6].

2.2.2. Determination of fecal SCFA concentrations

We measured three kinds of fecal SCFAs (butyrate, propionate, and acetate) by high-performance liquid chromatography (HPLC). The amount of feces required for this measurement was 1 g. The concentration of each fecal SCFA was evaluated weekly for 6 weeks after patient admission.

Feces were homogenized in 1 ml distilled water, and the homogenate was placed in an Eppendorf tube and centrifuged at 10,000 rpm at 4 °C for 10 min. A mixture of 0.9 ml of the resulting supernatant and 0.1 ml of 1.5 mol/L perchloric acid was mixed well in a glass tube and allowed to stand at 4 °C for 12 h. The suspension was then passed through a filter with a pore size of 0.45 μm (Millipore Japan Ltd., Tokyo, Japan). The sample was analyzed for organic acids by HPLC as previously

Table 1 – Patient characteristics.

Case	1	2	3	4	5
Age (years)	50	71	25	28	37
Sex (M/F)	M	M	F	F	F
Burn index	75	30	30	35	60
Prognostic burn index	125	101	55	63	97
Operation frequency	7	1	5	2	5
Enteral nutrition	Continued	Continued	Continued	Continued	Stopped on day 38
Enteritis (Y/N)	Y	Y	Y	Y	N
Pneumonia (Y/N)	Y	N	Y	N	Y
Bacteremia (Y/N)	Y	N	N	N	Y
Prognosis	Survival	Survival	Survival	Survival	Death