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Effect of Enteral Nutrition on Intestinal Permeability in Critically Ill Patients —Preliminary Report—

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Abstract It has been reported that intravenous injection of endotoxin increases intestinal permeability in human, and that total parenteral nutrition promotes bacterial translocation from the gut. In the present study, the effect of enteral nutrition on intestinal permeability and its relationship to plasma endotoxin levels were evaluated. Intestinal permeability was assessed in six stressed patients who were intubated for acute respiratory failure. The examination was performed before and after 7 days of enteral feeding using the dual-sugar intestinal permeability test, with lactulose and mannitol as markers. Plasma endotoxin levels were also measured by the endotoxin-specific colorimetric limulus test before and after an enteral diet. The mean±SD lactulose to mannitol excretion ratio (L/M ratio), a permeability index, before enteral nutrition was 0.108± 0.111, which was extremely high compaired to reported value of healthy volunteers. This value was declined to 0.042±0.067 after enteral nutrition, although p value (0.068) was not reached statistical significance. The mean plasma endotoxin level, which may have been related to concomitant respiratory infection, was 10.08±2.80 pg/ml before, and 18.48± 12.39 pg/ml after enteral feeding (p=0.075). Despite the elevation of endotoxin, there was a tendency to make intestinal permeability improve after enteral feeding. This suggests that the increased progression of intestinal permeability due to endotoxemia, fasting, and acute stress might be prevented by enteral feeding.

Key words: Enteral nutrition, Intestinal permeability, Endotoxin, Endotoxemia, Lactulose-mannitol differential sugar absorption

Introduction

Bacterial translocation is defined as the passage of viable organisms through the epithelial mucosa into the lamina propria, to the mesenteric lymph nodes, and possibly to other tissues (1). It has been reported that intravenous injection of endotoxin in human volunteers increases intestinal permeability (2), and that total parenteral nutrition promotes bacterial translocation from the gut by increasing the cecal bacterial count and impairing intestinal defenses (3). It has also been reported, in experimental models, that bacterial translocation is promoted by endotoxin administration (4).

In the present study intestinal permeability was assessed in six stressed patients with respiratory failure. Examination was performed using the lactulose-mannitol clearance assay, a dual-sugar intestinal permeability test. The effect of enteral nutrition on intesti-

nal permeability and its relationship to plasma endotoxin levels were studied.

Materials and Methods

There were six patients (five men and one woman, aged 54 to 76 years, mean: 68.7 years) who were admitted to the Critical Care Medical Center at the Yamaguchi University Hospital between July 1, and August 31, 1994 for acute respiratory failure. Primary pulmonary disease for each patient is one due to radiation pneumonitis; one, postoperative state of ascending aortic aneurysm; two, postoperative state of neurosugery; and two, bacterinal pneumonia. All patients were intubated on admission to the center. The APACHE II(Acute Physiology and Chronic Health Evaluation) score without the Glasgow coma scale was recorded to assess the severity of illness.

The effect of enteral nutrition on intestinal permeability was measured using the dual, non-metabolized sugar absorption test. Lactulose and mannitol were used as permeability markers (5,6). Plasma endotoxin levels were determined, and arterial blood and sputum were obtained for culture.

After at least 7 days of parenteral nutrition (range: 7 to 11 days, mean: 8.5 days), an enteral diet (Twinline®, Otsuka pharmaceutical Co. Ltd., Japan) was administered for the subsequent 7 days through a nasoenteral tube, the tip of which had been placed beyond the Treitz's ligament endoscopically, and confirmed fluoroscopically. Twinline® consists of protein, fat, carbohydrate, vitamins, minerals, and electrolytes (Table 1). During the first two days, 400ml of Twinline® and 400ml of water were mixed and administered continuously at 33ml/hr. During the next 2 days, full-strength Twinline® was administered continuously at 33ml/hr. During the final 3 days, full-strength Twinline® was administered continuously at 55ml/hr.

Before and just after 7 days of enteral nutrition period, the patients received 12g of lactulose and 5g of mannitol dissolved in 100ml of water via the nasoenteral tube. Urine was collected for the next 6 hours and the samples were stored at -80℃ until analysis. Urinary lactulose and mannitol concentrations were determined using capillary gas chromatography (SRL Co. Ltd., Japan). The amount of each sugar excreted in the urine during the 6 hours was then converted to a percentage of the amount of the sugar that had been administered enterally. The fraction of lactulose excreted was indexed to the mannitol excretion fraction by dividing the lactulose excretion fraction by the mannitol excretion fraction, yielding a permeability index, the lactulose/mannitol excretion ratio (L/M ratio).

Table 1. Compositions and Components in 400ml/400Kcal of Enteral Diet

Protein	16.2 g*	Pantothenic acid	3.76 mg
Carbohydrate	58.72 g	Folic acid	100 μg
Fat	11.12 g	Biotin	15.6 μg
Retinol palmitate	828 IU	Sodium	276 (12.0) mg (mEq)
Cholecalcifenol	54 IU	Potassium	472 (12.1) mg (mEq)
Tocopherol acetate	2.68 mg	Calcium	176 (8.8) mg (mEq)
Phytonadione	0.252 mg	Magnesium	56 (4.6) mg (mEq)
Thiamine	808 μg	Phosphorus	212 (6.8) mg (mmol)**
Riboflavin	900 μg	Chloride	42 (12.1) mg (mEq)
Pyridoxine	992 μg	Iron	2.52 mg
Cyanocobalamin	1.28 μg	Zinc	3.78 mg
Ascorbic acid	90 mg	Manganese	640 μg
Nicotinamide	9.92 mg	Copper	92 μg

* calculated from nitrogen content
** analyzed value (mean of seven lots)

Blood samples were collected for measurement of endotoxin levels, and for bacterial cultures before and just after 7 days of enteral nutrition. Platelet-rich plasma was prepared from the blood by centrifugation at 3000 rpm for 40 seconds. Endotoxin levels were determined using the endotoxin-specific colorimetric limulus test (Endospecy Test, Seikagaku-kogyo, Japan), and diazo-coupling in plasma treated with perchloric acid (PCA) as described by Inada et al. (7). The sensitivity of this new PCA method was found to be about eight times greater than that of conventional PCA methods. An endotoxin level greater than 9.8 pg/ml was determined to be abnormal in healthy volunteers (7).

Sputums were obtained for culture through the endotracheal tube before and just 7 days after enteral feeding.

None of the patients had a history of gastrointestinal resection, primary gastrointestinal disease, or renal disease.

The results are reported as the mean±SD. Statistical comparisons were performed using the Wilcoxon signed-rank test, with a $p<0.05$ considered significant.

Results

Table 2 and Table 3 show the results of intestinal permeability and bacteriology. The mean APACHE II score was 7.7±3.4 before enteral nutrition, and 9.0±3.9 after enteral nutrition ($p=0.129$). Patients excreted 0.29±0.31% and 0.18±0.30% of the lactulose administered ($p=0.068$), and 11.38±6.98% and 7.59±3.62% of the mannitol administered ($p=0.600$), before and after enteral nutrition, respectively. The mean L/M ratio was 0.108±0.111 before, and 0.042±0.067 after 7 days of enteral feeding ($p=0.068$). The mean plasma endotoxin level was 10.08±2.80 pg/ml before, and 18.48±12.39 pg/ml after enteral nutrition ($p=0.075$). Blood cultures revealed *Staphylococcus capitis*, a gram-positive organism, in one patient before enteral feeding. In the sputum, a variety of gram-negative organisms were detected. Of the six patients in this study, four were discharged from the unit, and two died.

Discussion

The dual-sugar intestinal permeability test has been used to assess small bowel mucosal

Table 2. Patient Characteristics, and Results of Intestinal Permeability and Bacteriology before and after Enteral Nutrition

Patient No.		APACHE II score	Lactulose (μg/ml)	%Lactulose excretion	Mannitol (μg/ml)	%Mannitol excretion	L/M ratio	Endotoxin (pg/ml)	Bacterial Culture Blood	Bacterial Culture Sputum
1†	Pre	12	37	0.28	1099	19.78	0.03	11.0	negative	*Xanthomonas maltophilia*
72 male	Post	14	0	0.00	314	5.46	0.00	11.8	negative	*Pseudomonas fluorescens*
2	Pre	2	83	0.82	330	7.79	0.25	11.8	negative	negative
54 male	Post	2	103	0.76	604	10.63	0.17	18.0	negative	
3†	Pre	7	54	0.20	337	14.81	0.16	13.3	negative	*Pseudomonas aeruginosa*
71 female	Post	10	13	0.12	566	12.85	0.02	43.0	negative	
4	Pre	10	0	0.00	752	17.75	0.00	7.8	*Staphylococcus capitis*	*Pseudomonas aeruginosa*
71 male	Post	8	0	0.00	144	5.01	0.00	15.7	negative	*Alcaligens xylosoxidans*
5	Pre	7	0	0.00	255	3.26	0.00	10.9	negative	*Pseudomonas aeruginosa*
68 male	Post	10	0	0.00	125	3.46	0.00	9.2	negative	*Candida tropicalis*
6	Pre	8	100	0.44	466	4.89	0.21	5.7	negative	*Pseudomonas aeruginosa*
76 male	Post	10	22	0.21	347	8.12	0.06	13.2	negative	*Alcaligens xylosoxidans*

† Dead soon after study

Table 3. Mean Total Levels of Each Marker before and after Enteral Nutrition

	APACHE II score	%lactulose excretion	%mannitol excretion	L/M ratio	Endotoxin (pg/ml)
Pre	7.7±3.4	0.29±0.31	11.38±6.98	0.108±0.111	10.08±2.8
Post	9.0±3.9	0.18±0.30	7.59±3.62	0.042±0.06	18.48±12.39
p value	0.129	0.068	0.6	0.06	0.07

function and integrity, instead of endoscopy and biopsy (5). It is sensitive, low cost, and simple to perform (6). This test is based on the different absorption routes of the two sugars. Mannitol, a monosaccharide, passes through aqueous pores in the cell membrane (the transcellular route), while lactulose, a disaccharide, is absorbed via extrusion zones at the villous tips, and at tight junctions (the paracellular route)(8,9). Normally, 10 to 20% of the mannitol administered enterally is absorbed. On the contrary, lactulose is poorly absorbed enterally. Alterations in absorption of lactulose reflect mucosal leakiness, whereas decreased absorption of mannitol correlates with a decrease in functional absorptive area (6,10). Mucosal damage leading to altered intestinal permeability has a greater effect on lactulose absorption and subsequent renal excretion than on mannitol absorption. By indexing the excretion of lactulose to that of mannitol, it is possible to correct for variables unrelated to intestinal permeability such as gastric emptying, intestinal transit time, mucosal surface area, cardiac output, renal clearance, and accuracy of urine collection. Although the results obtained from a single marker of permeability may be altered by these factors, the absorption ratio of two markers will not be altered because both markers will be equally affected.

In the present study, intestinal permeability was assessed in critically ill patients under severe stress. The effect of enteral nutrition on intestinal permeability and its relationship to plasma endotoxin levels were studied. In recent studies the L/M ratio in healthy controls has been reported to range from 0.008 to 0.035 (7,9,10,11,12).

No significant correlation was found between the mean L/M ratio and the severity of illness demonstrated by APACHE II scores. The mean L/M ratio was 0.108±0.111 in stressed patients with acute respiratory failure before enteral feeding, suggesting that intestinal permeability was increased. In the literature, the L/M ratio has been reported to range from 0.113 to 0.159, even in burn patients (9,10).

Berg and Garlington (1) have defined bacterial translocation as the passage of viable organisms through the epithelial mucosa into the lamina propria, to the mesenteric lymph nodes, and possibly to other tissues. Bacterial translocation may result from loss of the effective intestinal mucosal barrier (11). However, few studies have evaluated how microbes pass through the intestinal barrier. Cole et al.(14) have demonstrated direct candidal invasion of enterocytes and Alexander et al.(15) have reported that microbial translocation occurs by direct penetration of the enterocyte, and that translocation does not require loss of mucosal integrity. Deitch et al. (16) have observed invasion by *Escherichia coli, Proteus, Pseudomonas, Enterococcus* and *Staphylococcus* in edematous mucosa which exhibited separation of the epithelium from the basal lamina at the villous tip following hemorrhagic shock. Wells et al.(17) have

reported that the intestinal macrophages may play a key role in the transport of intestinal particles (including bacteria) to extra-intestinal sites, and have recently reported (18) that *Enterococcus faecalis* can translocate across an intact intestinal tract with intestinal overgrowth.

Total parenteral nutrition promotes bacterial translocation from the gut by increasing the cecal bacterial count, and impairing the intestinal defenses (3). Experimentally, bacterial translocation is promoted by endotoxin administration. In addition, the gut has been implicated as a reservoir for bacteria causing systemic infections during endotoxemia (2,4). O'Dwyer et al.(2) have reported that intravenous injection of endotoxin in human volunteers increases intestinal permeability and that lactulose excretion is more affected than mannitol excretion by endotoxin injection. It is not clear whether increased intestinal permeability caused the infections, or whether the infections caused disruption of the gut barrier.

Although we detected endotoxin higher than that of the prior value before enteral nutrition, we speculate that it might have originated from a worsen respiratory infection. There was a tendency to improve intestinal permeability after enteral feeding, which was resulted from improvement of the percentage lactulose excretion and the L/M ratio. These findings suggest that the progression of intestinal permeability due to endotoxemia, fasting, and acute stress might be prevented by administration of enteral feeding. More intensive prospective randomized trial will be indicated to confirm this potential therapeutic benefit.

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