INTRODUCTION

Bacterial translocation (BT) is defined as the passage of viable indigenous bacteria from the gastrointestinal tract to normally sterile extraintestinal sites, such as the mesenteric lymph nodes, spleen, liver, peritoneal cavity and bloodstream (1). It is known that BT from the gut mucosa in patients with sepsis, burn, or trauma causes the systemic sepsis, ultimately leading to multiple organ failure (2, 3).

Glutamine (GLN), the most abundant free amino acid in the circulation, is the primary fuel for cell division and proliferation of the intestine and plays a key role in the transport of nitrogen between organs (4, 5). Recent studies indicated that enteral or parenteral GLN reduces BT, and maintains the gut mucosal integrity (6-8), though there have been some controversies about the effect of GLN on BT (9, 10). Growth hormone (GH), a strong anabolic hormone, has been reported to show the prevention of nitrogen loss and the protein sparing effect when it was administered to the patients with sepsis, burn, or trauma causes the systemic sepsis, ultimately leading to multiple organ failure (2, 3).

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In this study, we investigated the combined effect of glutamine (GLN) and growth hormone (GH) on bacterial translocation (BT) in sepsis. After single intraperitoneal injection of lipopolysaccharide (10 mg/kg), 48 rats were divided randomly into four groups of 12 animals each: the control group received chow orally; the GLN group received chow plus 10% GLN; GH group received chow plus GH; and the GLN/GH group received chow, 10% GLN, and GH. Twenty-four and 96 hr later, rats were sacrificed. Portal blood culture, bacterial colony counts of cultured mesenteric lymph nodes, mucosal thickness, malondialdehyde (MDA), and glutathione (GSH) levels in the gut mucosa were measured. There was no significant change of the rate of portal blood culture between all treatment groups at 24 and 96 hr. At 24 hr, the rats receiving combined treatment of GLN and GH showed lower bacterial colony counts and mucosal MDA levels than the control rats, and higher mucosal GSH levels than the control and GLN-treated rats. At 96 hr, rats treated with both GLN and GH exhibited lower bacterial colony counts and mucosal MDA levels, and higher mucosal thickness and GSH levels than control, GLN, or GH-treated rats. This study suggests that the combination of GLN and GH may synergistically reduce BT over time in sepsis.

Key Words: Glutamine; Growth Hormone; Bacterial Translocation; Sepsis

MATERIALS AND METHODS

Experimental preparation

This study was approved by the Clinical Research Institute and Laboratory for Experimental Animal Research. Forty eight specific pathogen free male Sprague-Dawley rats weighing 280-310 g were kept with a 12 hr light-dark cycle and allowed ad lib access to chow and water. Animals were fasted 8 hr before the experiment, and anesthetized with an intramuscular injection of 5 mg of ketamine hydrochloride/100 mg of body weight. To make a sepsis model, each rat was given a single intraperitoneal injection of 10 mg/kg of lipopolysaccharide (LPS, Sigma). After LPS treatment, all rats were administered with
Study design

The rats were assigned to one of the four groups (n=12 in each group) after LPS treatment: the control group received chow and water; the GLN group received chow and water and additionally was given 2 mL of 10% GLN via a metal gavage into the stomach every 8 hr; the GH group received chow and water and intramuscular injection of 2.0 L.U./kg/day of GH (recombinant human GH, LG chemistry, Korea); and the GLN/GH group received chow and water, 2 mL of 10% GLN every 8 hr, and 2.0 L.U./kg/day of GH. Six animals in each group were anesthetized with ketamine hydrochloride (7.5 mg/100 mg intramuscularly) at 24 and 96 hr following LPS treatment, respectively. After the abdominal cavity was opened, the mesenteric lymph nodes were removed aseptically and placed in the ice box. The distal 30 cm of the terminal ileum proximal to the ileocecal valve was rapidly removed, and the distal 2 cm of the terminal ileum was put into 4% paraformaldehyde solution for the measurement of the mucosal thickness. The remaining ileum was longitudinally opened, and thoroughly washed with cold 0.9% saline. To separate the mucosa from the muscle and serosa of the intestine, the removed terminal ileum was scraped on cold slide glass using another slide glass. Separated mucosa tissues were rapidly frozen between two blocks of dry ice and stored at -70°C for subsequent biochemical assays.

Mesenteric lymph node and portal blood cultures

To determine bacterial colony counts, the mesenteric lymph nodes were aseptically and carefully obtained. The fresh mesenteric lymph nodes were weighed and homogenized aseptically after 0.5 mL of 0.9% saline was added. Aliquots (0.2 mL) of each of the homogenized lymph nodes solution were plated on blood and McConkey agar culture plates. All culture plates were aseptically incubated at 37°C in the clean bench and examined for growth at 24 and 48 hr. The values of mean colony counts obtained from the agar plates were expressed as the colony-forming unit per gram of the mesenteric lymph nodes (CFU/g of mesenteric lymph nodes). When the abdominal cavity was opened, portal blood (0.2 mL) was collected under the microscope, and cultured aerobically and anaerobically using BAC-TEC broth and Thioglycollate broth, respectively. Blood cultures were continuously monitored for 7 days. When the culture broth looked turbid, organisms in the culture broths were identified by standard bacteriological techniques using blood and chocolate agar plates.

Measurement of mucosal thickness

Samples of the intestinal tissues were fixed in 4% paraformaldehyde and dehydrated with alcohol and then paraffin-embedded. The formatted specimens were cut by sliding microtome and stained with hematoxylin and eosin. Five slide samples were made in each intestinal sample and the mucosal point with the longest villi in each slide was measured. Morphometric measurements were performed using an image analyzer (Image-Pro Plus). Slide samples were randomly chosen for each animal and examined under oil using an Olympus BH2 microscope. For each animal, 20 randomly chosen mucosal regions were traced. The thickness of the gut mucosa was expressed as the mean of these measurement values (μm).

Biochemical assays

To assess the mucosal integrity indirectly, gut lipid peroxidation and antioxidant defenses were measured. Malondialdehyde (MDA), a byproduct of lipid peroxidation, was measured by the method of Ohkawa et al. using the thiobarbituric acid (18). The level of MDA was presented as nmol/g of tissue. Gut GSH content, antioxidant defenses, was measured according to the DTNB-GSSG Reductase Recycling Assay of Griffith (19), which provides a very sensitive assay for total tissue glutathione as described by the following equation.

\[
\text{GSH}= \text{total glutathione} - 0.5 \times \text{GSSG (oxidized glutathione)}
\]

Values were expressed as μmol/g of tissues.

Statistical analysis

SPSS for Windows release 10.0 was used for the statistical analysis. The statistical significance among the mean values in the four treatment groups was determined by nonparametric Kruskal-Wallis test. Mann-Whitney U test was used to compare the mean values in two treatment groups. The values were expressed as mean ± standard deviation. Chi-square test was also done to compare the portal blood culture rates between the treatment groups. Nonparametric Spearman’s correlation test was used to determine the correlation between the measured parameters. A p value of <0.05 was considered to be statistically significant.

RESULTS

Portal blood culture and bacterial colony count of mesenteric lymph node culture

All organisms in positive portal blood cultures were Escherichia coli. There were no significant changes of the positive culture rates of the portal blood between all treatment groups at 24 and 96 hr, respectively: control (3/6) vs GLN (2/6) vs GH (2/6) vs GLN/GH (0/6) at 24 hr (p=0.280) and control (2/6) vs GLN (0/6) vs GH (0/6) vs GLN/GH (0/6) at 96 hr (p=0.088), respectively. There was a significant decrease in bacterial colony counts in the GLN, GH, and GLN/GH groups compared with the control group at 24 hr (p=0.026, p=0.004,
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At 96 hr, both the GLN/GH (p=0.002) and GH (p=0.009) groups showed a significant decrease in bacterial colony counts compared with the control group. There was also a significant decrease in bacterial colony counts at 96 hr in the GLN/GH group compared with the GLN (p=0.041) and GH (p=0.041) groups (Fig. 1A).

Mucosal thickness

There were no significant changes of mucosal thickness between treatment groups at 24 hr. At 96 hr, mucosal thickness was significantly increased in the GLN/GH (p=0.002) and GH (p=0.009) groups compared with the control group. The GLN/GH group also showed a significant increase in mucosal thickness compared with the GLN and GH groups at 96 hr (p=0.015, p=0.009, respectively) (Fig. 1B).

Gut mucosal MDA and GSH levels

While the mucosal MDA level at 24 hr was significantly decreased in the GLN/GH compared with the control group (p=0.002), it was significantly decreased in the GLN (p=0.015) and GH (p=0.002) groups as well as in the GLN/GH (p=0.002) group compared with the control group at 96 hr. The GLN/GH group also showed lower levels of mucosal MDA than the GLN (p=0.009) and GH (p=0.041) groups (Fig. 2A).

There was a significant increase in the mucosal GSH level in the GLN/GH and GH groups at 24 hr compared with the control (p=0.002, p=0.002, respectively) and GLN groups (p=0.004, p=0.002, respectively). While the GH group showed a significant increase in the mucosal GSH level at 96 hr compared with the control group (p=0.002), the GLN/GH group showed a significant increase in the mucosal GSH level compared with the control (p=0.002), GLN (p=0.041), and GH groups (p=0.041) (Fig. 2B).

Correlation between parameters

There was a correlation between the bacterial colony count and mucosal thickness. Significant correlations were also observed between the colony count and gut mucosal GSH content, and between the mucosal thickness and the gut GSH content at 96 hr. The gut mucosal MDA level showed correlations to the colony count, mucosal thickness, and gut GSH content (Table 1).
**DISCUSSION**

Glutamine (GLN), either in enteral or parenteral form, increases the intestinal villous height, maintains the mucosal integrity, and reduces BT (4). Animal studies have shown that GLN-enriched diets show a decreased BT and an improvement in the ability to kill translocated *E. coli* and survival in rats with gut-origin sepsis (6, 8). In this study, however, GLN only treatment was not as effective as the combined treatment of GLN and GH or GH only for the prevention of BT in septic rat. The decreased effect of GLN seems to be caused by gut GLN depletion resulting from the impaired intestinal GLN uptake induced by sepsis. Previous studies have shown that sodium-dependent GLN transport by enterocyte decreased in sepsis (20, 21). This decrease can be caused by reduced availability of circulating substrate for synthesis of carrier proteins by enterocyte, because sepsis causes a decrease in mesenteric blood flow in the small intestine. In contrast, GH appears to increase the intestinal GLN uptake and to induce hepatic release of GLN in short bowel syndrome or sepsis (22-24). It has been shown that GH increases intestinal GLN uptake induced by system B amino acid transport, and this upregulation is due, in part, to an increase in GLN carrier capacity (25).

In previous studies, the combined treatment of GLN and GH has been reported to initially improve the absorption of protein, increase body weight and lean body mass, decrease body fat, and decrease stool output in patients with short bowel syndrome (16). In contrast, other studies showed that the combination of GLN and GH did not enhance mucosal mass, mucosal protein, or mucosal DNA level in rats receiving massive small bowel resection (26), nor improved intestinal absorption, mucosal morphology, or stool losses in patients with short bowel syndrome (27, 28). However, we showed that the combined treatment of GLN and GH was the most effective on BT. The synergistic effect of the combination of GLN and GH shown in this study may result from the similar mechanism as noted above. Despite septic condition, gut GLN uptake would increase markedly if GH was given simultaneously with the administration of GLN. The mechanism by which the combination of GLN and GH exerts the synergistic effect on BT appears to result from increased gut GLN uptake by GH. Unneberg et al. reported a similar result in an animal trauma model (17).

MDA, a byproduct of lipid peroxidation, is considered to be an indicator of tissue lipid peroxidation by oxygen free radicals. Lipid peroxidation of gut mucosa can result in the disruption of the mucosal barrier function followed by endotoxemia and BT (29), and that there might be an association between BT and intestinal mucosal lipid peroxidation (30). In our study, the gut MDA level showed positive correlation to the bacterial colony count and negative correlation to the gut mucosal thickness. This result suggests that there is an association between the gut MDA level and BT, and that the gut MDA level may reflect the disruption of gut mucosal integrity indirectly. Therefore, the reduced gut MDA level in GLN, GH, or the combination of GLN and GH treatment in our study indicates that these treatments decrease gut lipid peroxidation, maintain gut mucosal integrity, and reduce BT.

GH, a scavenger of reactive oxygen free radicals, protects tissues from reactive oxygen free radicals-mediated cell injury (31). We found that GH only or the combination treatment of GLN and GH significantly increased gut GSH contents, but GLN only treatment did not show an increase in the gut GSH content. These two treatment groups also showed higher gut GSH contents than the GLN treatment group. These results suggest that GH may be more effective for the increase of gut GSH than GLN. GH has been reported to increase plasma GSH in multiple trauma patients (32), and to increase lung and liver GSH in burned rats (33). GLN, a component of GSH, enhances gut GSH production (34) and maintains gut GSH levels during bowel ischemia/reperfusion (31). As noted earlier, gut GLN uptake decreases in sepsis, while GH increases gut GLN uptake. Therefore, GH appears to increase gut GSH levels by increasing gut GLN uptake.

In this study, we measured mucosal thickness instead of mucosal integrity to determine BT. Although there is some association between mucosal integrity and mucosal thickness, mucosal integrity is different from mucosal thickness. Several studies showed that increased mucosal mass (thickness) did not reduce BT (9, 10). Loss of mucosal integrity has been proposed as promoting bacterial translocation, following administration of endotoxin (35). Although we measured the gut MDA level to assess the extent of the mucosal disruption indirectly, we do not think that the gut MDA level reflected the extent of mucosal integrity accurately. For assessment of

### Table 1. Correlation between parameters

| Parameters                     | r (24 hr) | p value  | r (96 hr) | p value  |
|-------------------------------|----------|----------|-----------|----------|
| Colonies counts-Mucosal thickness | r=-0.556 | p=0.005  | r=-0.803  | p<0.001  |
| Colonies counts-Gut MDA       | r=0.774  | p<0.001  | r=0.472   | p=0.02   |
| Colonies counts-Gut GSH       | r=0.527  | p=0.008  | r=0.588   | p=0.002  |
| Mucosal thickness-Gut MDA     | r=-0.492 | p=0.015  | r=0.674   | p=0.001  |
| Mucosal thickness-Gut GSH     | -        | p=0.033  | r=0.752   | p<0.001  |
| Gut MDA-Gut GSH               | r=-0.436 | p<0.001  | r=-0.719  | p<0.001  |

r: correlation coefficient.
mucosal integrity, further measurements including intestinal cellular proliferation or intestinal permeability test (transmucosal resistance, mannitol, lactulose, polyethylene glycol flux, and so on) seem to be needed.

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