Juzentaihoto reduces post-partial hepatectomy hyperammonemia by stabilizing intestinal microbiota

Yoshihiro IMAZU,1,a) Kenji TSUI,1,b,c) Takahiro TODA,1,c,d) Atsushi ISHIGE,2) Kiyoshi SUGIYAMA,3) Yoshimi BENO,2) Kenji WATANABE,1,a) Masaki KITAIMA1,a,b)

1Department of Kampo Medicine, Keio University, School of Medicine, 35 Shinano-machi, Shinjuku-ku, Tokyo, 160-8582, Japan.
2Department of Surgery, Keio University, School of Medicine, 35 Shinano-machi, Shinjuku-ku, Tokyo, 160-8582, Japan.
3Department of Surgery, Saiseikai central hospital, 1-4-17 Mita, Minato-ku, Tokyo, 108-0073, Japan. 4Department of Clinical Pharmacokinetics, Hoshi University, School of Pharmacy, 2-4-41 Ebara, Shinagawa-ku, Tokyo, 142-8501, Japan. 5Japan Collection of Microorganisms, RIKEN BioResource Center, 2-1 Hirosawa, Wako, Saitama, 351-0198, Japan. (Received October 2, 2006. Accepted November 20, 2006.)

We examined the preventive effect of the Kampo medicine Juzentaihoto (JTX) on post-partial hepatectomy-induced hyperammonemia, a frequent and potentially fatal consequence of this surgery for resection of hepatocellular carcinomas. In most cases, these liver tumors are associated with pre-existing liver damage such as cirrhosis or chronic hepatitis. Post-surgical hyperammonemia harms further the remaining liver as well brain and other vital functions. With pre-surgical JTX administration, this post-surgical hyperammonemia is suppressed significantly. To explain this phenomenon, we first hypothesized that JTX prevents further damage of the liver, a site of ammonia metabolism. However, post-surgical liver dysfunction is not improved with JTX. Thus we focused on the other source of ammonia, the intestinal microbiota, as the source of the hyperammonemias. To examine the possible effect of JTX on intestinal microbiota, terminal restriction fragment polymorphism (T-RFLP), a culture-independent microbial analysis, was used to document change in the intestinal microbiota with JTX. We documented that partial hepatectomy changed the intestinal microbiota. Then we demonstrated that with oral JTX administration, this post-surgical change of the intestinal microbiota was not observed even after partial hepatectomy. We also showed that the representative ammonia-producing bacteria, Bacteroides, increased with partial hepatectomy and decreased with JTX administration. Cluster analysis of fecal microbiota suggests that JTX administration stabilized the intestinal microbiota and maintained the post-surgical microbial analysis environment of the gut. This study suggests that JTX is useful to prevent the clinically significant increase in the serum ammonia levels after partial hepatectomy.

Key words Kampo medicine, Juzentaihoto (JTX), intestinal microbiota, terminal restriction fragment length polymorphism (T-RFLP), hyperammonemia, hepatectomy.

Introduction

Worldwide, millions of persons are estimated to have chronic hepatitis due to either the hepatitis B or C viruses. Such chronic infections are a significant risk factor for both liver cirrhosis and hepatocellular carcinoma. Even though the only curative therapy for hepatocellular carcinoma is surgical excision, this is quite difficult and many complications need to be considered.

Hyperammonemia, one of the major operative complications of partial hepatectomy, is associated with significant morbidity and mortality. Increased serum ammonia levels cause brain edema,1,2 encephalopathy,1,2 and further deterioration in liver function.3 Therapeutic strategies to suppress blood ammonia level, therefore, are the mainstay in the prevention and treatment of hyperammonemia. Various forms of therapy have been tried, i.e. gut cleansing, branched chain amino acid perfusion, lactulose or neomycin administration, plasmapheresis, hemodialysis or charcoal hemoperfusion. Despite these enthusiastic trials, controlled studies have not yet provided the clear evidence of the prevention or treatment of hyper-ammonemia after partial hepatectomy.3,4 Thus, a new prevention or treatment modality is needed.

Previously, we have shown that the traditional Japanese herbal medicine Juzentaihoto (JTX) changed the intestinal microbiota (in press). Currently JTX is already widely prescribed for the prevention of both cancer metastases as well as infection in immuno-compromised patients.4,5 The potential for additional benefit from JTX in patients with a history of liver cancer is compelling.

In this present study, we have shown that the oral administration of JTX suppressed significantly the hyperammonemia frequently seen after partial hepatectomy without restoration or protection of liver function. We analyzed the intestinal microbiota and have shown that the partial hepatectomy itself disturbed and changed the intestinal microbiota. This disturbance was inhibited with JTX administration. Thus, stabilization of the intestinal microbiota appears to be the mechanism of action by which JTX suppresses post-partial hepatectomy hyperammonemia.
Materials and Methods

Animals. Male BALB/c mice (CLEA Japan Inc., Kawasaki) aged 7 to 9 weeks and weighing 20-25 grams were used in each of the experiments. They were housed in an air-conditioned room (temperature 24 ± 1 °C) with a controlled light/dark cycle (light on between 6:30AM and 7:00PM) with food and water available ad libitum both before and after the experimental procedures. This protocol was approved by the Guidance for the Care and Use of Laboratory Animals of Keio University School of Medicine that is in accordance with the NIH Guide for Care and Use of Laboratory Animals.

Sample preparations. Juzentaihoto (JTX) (Tsumura & Co., Tokyo) is a standardized prescription traditional medicine that consists of 10 herbs in a fixed ratio. The ingredients were extracted by hot water method and processed into granules by a proprietary method.

A granule of JTX (1.0 g) was extracted with methanol (20 mL) under ultrasonication for 30 min., and was centrifuged at 3000rpm for 5 min. The supernatant was filtrated with a membrane filter (0.45 μm) and then submitted for HPLC analysis (30 μL).

3D-HPLC analysis. HPLC apparatus consisted of a Shimadzu LC 10A (analysis system software: CLASS-M10A ver. 1.64, Tokyo, Japan) equipped with a multiple wavelength detector (UV 200-400 nm)(Shimadzu SPD-M10AVP, diode array detector), an auto injector (Shimadzu CTO-10AC). HPLC conditions were described as follows: column, ODS (TSK-GEL 80TS, 250 × 4.6 mm i.d., TOSOH, Tokyo, Japan); eluant, (A) 0.05M AcONH4 (pH 3.6) (B) 100% CH3CN. A linear gradient of 90% A and 10% B changing over 60 min to 0% A and 100% B was used. (And 100% B was continued for 20 min.); temperature,40temp. degree; flow rate, 1.0mL/min.

Compared with the Juzentaihoto and water in hepatectomy mice. The mice were randomly divided into three groups of six mice: 1) control, 2) partial hepatectomy only, and 3) partial hepatectomy plus JTX. The Juzentaihoto (Tsumura & Co., Tokyo, Japan) was dissolved in aqueous solution (1.0 g/kg body weight) and administered using a stainless steel gastric tube once a day for 7 days before and 3 days after partial hepatectomy. Control and comparison mice were treated with an equal volume of water.

For the operated mice, a two-thirds partial hepatectomy was performed under anesthesia with an intraperitoneal injection of pentobarbital (50 mg/kg body weight). The fecal condition was observed daily.

Measurement of blood chemistry. Serum samples for measurement of GOT, GPT, T-Bil, BUN and ammonia levels were collected from the caudal vein before and after administration of the JTX and both 1 and 3 days after partial hepatectomy. These serum values were measured using DRY CHEM (FUJIFILM medical. Co. Ltd, Tokyo).

Sampling of fecal extracts. Fecal samples from mice were taken before administration of JTX until 3 days after partial hepatectomy. Collected samples were stored immediately at -80 °C until use.

Cell lysis and DNA isolation from samples. The DNA was isolated from the fecal samples using QIA amp DNA Stool Mini Kit (QIAGEN KK, Japan, Tokyo).

T-RFLP analysis and sequencing. The primers used for the PCR amplification of 16S rDNA sequences were 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-GTT TAC CTT GTG ACC ACT T-3'). 27F was labeled at the 5' end with the 6-carboxyfluorescein (6-FAM) (Applied Biosystems Japan Ltd., Tokyo). PCR was performed according to Kibe et al., 2004. Purified PCR products were digested with 20 U of Msp I (Takara Bio Inc., Tokyo) in a total volume of 10 μl at 37 °C for 3 hours. GS-2500 ROX (Applied Biosystems Japan Ltd., Tokyo) was used as internal standard markers. The length of the terminal restriction fragments (T-RFs) were analyzed by electrophoresis on a model ABI PRISM 310 Genetic Analyzer (Applied Biosystems Japan Ltd., Tokyo) and GeneScan analysis software (Applied Biosystems Japan Ltd., Tokyo). Gene cluster 3.0 was used for establishing the dendrogram type.

Real-time PCR assays. Mouse intestinal Bacteroides spp levels were analyzed by relative quantitation with the 2^-ΔΔCt method using real-time PCR with an iCycler IQ (BioRad Laboratories Inc, Japan, Tokyo). All samples were normalized to values of 16S rRNA, and results expressed as degree of changes of threshold cycle value relative to controls. Primer sequences from 5' to 3' were as follows: group I Bacteroides (Forward: CTGAACCAGCCAACTGCG, Reverse: CGCAACACTTCCACAgCTTA), group II Bacteroides (Forward: GTGTGCTTGAAGCACG, Reverse: ATCAAGGCTGACTCTTGCT), 16S rRNA (Forward: ACCGGTCGACTCCTAC, Reverse: GAAGGCCTCTT CATAACG).

Nucleotide sequence accession number. The sequence data determined in the present study have been assigned DDRJ, EMBL and GenBank Accession.

Statistical Analysis. The results are expressed as the mean ± standard deviation. Results from the three groups (Fig.2) were compared using Student's t-test and the Scheffe multiple comparison test. Data were considered statistically significant at p < 0.05.

Figure 1. The change of serum ammonia levels after partial hepatectomy and effect of JTX, as described. *p values obtained from T-test for serum ammonia levels.
Results

**JTX reduced serum ammonia levels.** Before the partial hepatectomy, the serum ammonia levels were not different between group 2, the hepatectomy without JTX group, and group 3, the hepatectomy with JTX group. (Fig. 2). Additionally, the JTX administration had no obvious effect on the serum ammonia levels before partial hepatectomy. Surgery induced significant changes in serum ammonia levels for group 2 with the pre-levels being quite low (0.2 ± 8 µg/dl) and the post-surgical day one levels quite elevated at 56.5 ± 17.5 µg/dl and the day three levels further elevated at 90.8 ± 7 µg/dl (p < 0.05). In contrast, for group 3, the post-surgery serum ammonia levels were significantly lower at 20.8 ± 5.1 µg/dl, approximately 75% lower than the level for group 2 (p < 0.05).

**Influence of JTX on liver and renal function.** Serum GOT, GPT, T-Bil and BUN levels were measured three days after partial hepatectomy (Table 1). No differences between the three arms existed. To illustrate, the value of GPT in the non-operated control group 1 (18.6 ± 0.6 U/L) was significant lower than in either mouse group 2 (91.2 ± 12.1 U/L) (p < 0.05) or mouse group 3 (93.6 ± 11.2 U/L) (p < 0.05).

**JTX changed the fecal microbiota patterns by T-RFLP analysis.** The fecal extracts collected before administration of JTX until three days after partial hepatectomy demonstrated that the condition of the feces was stable. No diarrhea was observed.

The changes of the fecal microbiota in the group 3 JTX administrated mice were assessed by T-RFLP analysis (Fig. 3 A, B) using T-RFLP patterns of 16S rRNA genes from samples digested with Msp I. The minimum and maximum values of the ordinate are 0 to 500 fluorescence units except for the small intestine samples digested with Msp I. T-RFLP patterns of group 3 were similar to that of group 1, the non-operated group. However, the microbiota patterns for group 2 differed significantly from both mouse group 1 and group 3 in respect to the cluster analysis data between 200 and 300 base.

Dendrogram analysis of fecal microbiota of mice was based on T-RFLP Dotted line represents the boundary between the clusters for groups 1, 2 and 3. The samples were divided into two parties. Differences between the party of the hepatectomy without JTX group and the mixed party of the non-operated group and the hepatectomy with JTX group were significant (Fig.3 B).

**Real-time PCR.** These results expressed as degree of changes of threshold cycle value relative to controls provided evidence that the hepatectomy without JTX group (0.46 ± 0.1) was lower than the non-operated group (1.70 ± 1) and the hepatectomy with JTX (1.66 ± 0.6) for group I Bacteroides species: *Bacteroides caccae*, *B. fragilis*, *B. ovatus*, *B. theta iotaomicron* and *B. vulgatus* (Fig.4 A).
For group II Bacteroides species, Bacteroides eggerthii, B. uniformis, B. stercoris, mice in group 2, the hepatectomy without ITX group, demonstrated significantly higher counts (246.4 ± 36.3) than the mouse group 1 controls (19.6 ± 7.3) and the mouse group 3 ITX group (0.03 ± 0.01) (p < 0.05) (Fig 4 B).

The differences between the non-operated control group and the hepatectomy with ITX group were not significant for either group I or group II Bacteroides species. The value in the non-operated group 1 and the hepatectomy with ITX group were significantly lower than the hepatectomy without ITX group by group II Bacteroides species (p < 0.05).

Many phytolotypes in the Clostridium group were found in the present study. All clones were divided into 134 species or phytolotypes (Table 2).

Discussion

The partial hepatectomy in healthy mice resulted in significantly elevated levels of serum ammonia. These elevated serum levels of this known hepatotoxin, ammonia, influenced the remnant liver function as represented by the elevated serum transaminases. Clinically, such significant elevations of serum ammonia levels can be quite harmful.

Ammonia is produced from glutamate by glutamate dehydrogenase in the mitochondria of the liver. This ammonia is toxic to the body and, under normal conditions, is quickly metabolized to urea via the urea cycle. However, other sources exist. Chief among these is the ammonia generated in the gastrointestinal tract. Nitrogenous compounds in the colon, which include ingested proteins and secreted urea, are degraded by bacteria. The liberated ammonia is then absorbed into the portal circulation, where concentrations are 5- to 10-fold greater than in mixed venous blood.

The most active groups of ammonia producers are the gram-negative anaerobic and aerobic rods, Clostridia and Bacilli. Within the colon, gram-negative anaerobes greatly outnumber Clostridia and Enterobacteria which in turn outnumber Bacillus spp., suggesting that anaerobes such as Bacteroides species may be the major contributors to intestinal ammonia, rather than the gram-negative aerobes which have been previously assumed to be the main resource of ammonia.

Clinically, ammonia is very toxic to the central nervous system and overload of ammonia induces hepatic encephalopathy and even coma. Persistent hepatic encephalopathy is a well-described complication of liver dysfunction and is an important disorder that seriously impairs daily functioning and quality of life in patients with cirrhosis or who are post-partial hepatectomy.

Two mechanisms are responsible for post-partial hepatectomy encephalopathy. First, surgical resection of the liver, especially in these persons whose livers have reduced functioning due to concurrent hepatic disease, impairs the body's capacity to manage ammonia loads. Second, the post-surgical impairment of hepatic reticuloendothelial function of the liver significantly increases the risk for endotoxemia. Under normal conditions, endotoxin from the gut bacteria is transported to the liver via the portal vein and is disposed by the reticuloendothelium in the liver. However, decreased reticuloendothelial function increases the risk of endotoxemia. If present, endotoxemia generates a systemic catabolic response resulting in muscle proteolysis and accelerated release of glutamine from muscle stores which in turn further increases ammonia production.

In both cases, after partial resection of the liver, the body's impaired capacity to handle the ammonia loads results in high systemic ammonia concentrations and progressive ammonia intoxication.

Various methods of treatment have been tried to minimize this complication. Treatment with lactulose is of benefit and is considered in many centers to be the treatment of choice. Lactulose was introduced approximately 30 years ago as a therapy for hepatic encephalopathy based on the concept that the drug acidifies the contents of the colon and...
Table 1. Blood chemistry was measured in the non-operated group, the hepatectomy with JTX group and the hepatectomy without JTX group respectively. *p values obtained from Scheffe multiple comparison test for the serum GPT levels.

<table>
<thead>
<tr>
<th></th>
<th>he non-operated group (Group 1) (n=6)</th>
<th>The hepatectomy without JTX group (Group 2) (n=6)</th>
<th>The hepatectomy with JTX group (Group 3) (n=6)</th>
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</thead>
<tbody>
<tr>
<td>GOT (U/L)</td>
<td>74 ± 11.89</td>
<td>138.67 ± 22.27</td>
<td>109.6 ± 8.81</td>
</tr>
<tr>
<td>GPT (U/L)</td>
<td>18.67 ± 0.61</td>
<td>91.17 ± 12.06*</td>
<td>93.60 ± 11.16*</td>
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<tr>
<td>T-Bil (mg/dL)</td>
<td>0.217 ± 0.03</td>
<td>0.267 ± 0.04</td>
<td>0.26 ± 0.01</td>
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<tr>
<td>BUN (mg/dL)</td>
<td>17.06 ± 1.12</td>
<td>15.92 ± 0.51</td>
<td>15.32 ± 0.19</td>
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*p < 0.05

Table 2. Association of bacterial species, phyotypes and group.

<table>
<thead>
<tr>
<th>Clostridium cluster</th>
<th>Bacterial species or phyotypes</th>
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<tr>
<td></td>
<td>Uncultured bacterium clone THM-10</td>
</tr>
<tr>
<td>IV</td>
<td>Anaeroebilum pentosovorum, Butyrate-producing bacterium A2-165, Clostridium cellulose, Clostridium orbicinum, Eubacterium siraeum, Eubacterium desmolae, Faecalibacterium prausnitzii, Ruminococcus albus, Ruminococcus flavefaciens, Ruminococcus sp. CO47, Uncultured bacterium clone (N=10), Uncultured Clostridium sp. N06, Uncultured firmicute clone (N=11), Uncultured Ruminococcus sp. (N=4)</td>
</tr>
<tr>
<td>IX</td>
<td>Megasaundra elsdeni, Mitsukokia jalaludini, Selenomonas infelix, Selenomonas ruminantium, Veillonella dispar, Veillonella parvula, Uncultured bacterium clone HuCB85, Uncultured firmicute clone (N=2), Uncultured Megasaundra sp. NB2G7</td>
</tr>
<tr>
<td>XI</td>
<td>Clostridium felsineum, C. litusebunense</td>
</tr>
<tr>
<td>XV</td>
<td>Eubacterium barkeri</td>
</tr>
<tr>
<td>XVII</td>
<td>Catenobacterium mitsuokai, Lactobacillus vilitinus, Lactobacillus cateniformis, Uncultured firmicute clone NB2A5</td>
</tr>
<tr>
<td>XVIII</td>
<td>Clostridium cocleatum, Clostridium ramosum, Clostridium spiroforme, Uncultured bacterium clone JW2E12</td>
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favors both trapping of ammonium ion in the lumen and prevention of absorption. In the colon, lactulose is metabolized by bacteria to release lactic, acetic and other organic acids, thereby decreasing stool pH to approximately 5.5.15
In the treatment of hepatic cirrhosis, lactulose has side effects such as flatulence and diarrhea. Flatulence leads to results paralytic ileus, bacterial translocation and systemic endotoxemia.18,19 Diarrhea also causes dehydration and electrolyte imbalances which increase the complexity of post-surgical care.
Antibiotics with activity against urease-producing bacteria, such as neomycin,17,18 paromomycin19 or metronidazole,20 also reduce the production of intestinal ammonia and have proved to be of value. Vancomycin has also been used in patients with lactulose-resistant chronic encephalopathy.21 The efficacy of neomycin is similar to that of lactulose.17 However, these drugs also induce diarrhea and renal dysfunction, especially with continuous use over several months.21 Additionally, such use of antibiotics increases the risk of creating new resistant pathogenic bacteria strains.
Thus, there are many indications for a new therapy to prevent or treat post-partial hepatectomy-induced hyperammonemia. In this study, JTX suppressed significantly the post-surgical elevations of serum ammonia levels (Fig. 2). Because the liver is the main organ to metabolize ammonia to urea, we first monitored serum transaminases and bilirubin and found JTX did not restore liver dysfunction caused by the partial hepatectomy (Table1). This result suggested that there is another mechanism of JTX to decrease the elevated serum ammonia level. Thus we focused on the second mechanism of inducing hyperammonemia, ammonia production by the intestinal microbiota.
Kampo medicines may effect intestinal ecology by antibiotic or prebiotic mechanisms.22,26 Some constituent herbs include Cinnamomi cortex and Paoniae radix of JTX and their ingredients include cinnamic aldehyde and c-methoxyxycinnamic aldehyde which have also been reported to have antimicrobial effects26,27 as well as a putative stimulating effect on the growth of certain bacterial species.28 One of the mechanisms of action of Kampo medicines has been postulated to both modulate intestinal microbiota and to exert their beneficial effects through this bacterial modulation.
However, up to now, changes in fecal microbiota by the administration of Kampo medicines have not been detected. Two possible reasons for this are considered. First, the recent studies using DNA-based estimation have
suggested that 70-80% of the total intestinal bacteria are uncultrueable, so that conventional detection methods based on the bacterial cultures may miss significant changes in intestinal microbiota. 29,30 Second, Kampo medicines contain many species of prebiotic compounds in relatively small quantities. The changes induced by the Kampo drug administration are assumed to be subtle and complex compared to those induced by a simple prebiotic compound such as oligosaccharide or starch.

We used T-RFLP analysis, which allows us to detect the change of fecal microbiota including currently unculturable bacteria (Fig.3 A). The partial hepatectomy changed the fecal microbiota pattern. This changed pattern was not observed with JTX administration in a pattern similar to that of the non-operated group. These observations were confirmed by the dendrogram, which divided the pattern into two clusters by the similarity of 72% (Fig.3 B). The results in the hepatectomy without JTX group (group 2) differed significantly from those of the non-operated group (group 1). In contrast, the results in the partial hepatectomy with JTX group (group 3) were similar to those in the non-operated group. The oral administration of JTX maintained the fecal microbiota pattern despite the surgical stress of the partial hepatectomy. This stabilization of the fecal microbiota may be the mechanism by which JTX decreases the serum ammonia level in partial hepatectomy.

The most active bacteria for ammonia production are anaerobic rods represented by the genus Bacteroides. 10 We detected members of the genus Bacteroides semi-quantitatively by PCR. The results confirmed that there was a difference between Bacteroides group I and group II. Bacteroides group I was decreased and group II was increased with JTX in hepatectomy groups. Even though both Bacteroides group I and group II produce ammonia, the influence by JTX behaves differently.

The representative fragments of the genus Bacteroides is around 100 bps in T-RFLP analysis. By the T-RFLP analysis, peaks of around 200 and 300 bps were changed significantly with hepatectomy. These peaks were referred to many known and unknown bacteria species (Table 2). These results confirmed that the changed T-RFLP fragment peaks were from miscellaneous microbiota strains and the change of the diverse intestinal microbiota caused the elevation of the serum ammonia level and difficult to attribute to one specific bacteria. Therefore, it is difficult to attribute hyper-ammonemia after partial hepatectomy to one or few specific microbiota. Thus, it is impossible to decrease the serum ammonia level with antibiotics targeting some specific microbiota.

It is known that the intestinal microbiota pattern returns to its baseline state after antibiotic administration or severe diarrhea disturbs thoroughly the host intestinal microbiota. 31,32 Even though the mechanism of how a certain pattern of intestinal microbiota recovers spontaneously is not clear, this fact suggests that the intestinal microbiota has a certain homeostatic mechanism to preserve the individual intestinal microbiota pattern. The intestinal microbiota pattern is different in each individual and is consistent over time. This pattern is determined by the mutual interaction between normal gut structure and function, and the bacteria. This commensal action has just started to be understood. 33 For example, when Bacteroides thetaiotaomicron was colonized in germ-free mice, many gene expressions in the epithelial cell were changed, such as genes dictating nutrient absorption, mucosal barrier function, xenobiotic metabolism, angiogenesis, the enteric nervous system and postnatal intestinal maturation. Furthermore, different bacterial species have been shown to elicit different changes in gene expressions and to participate in different physiological functions. 34

In the present study, this stabilizing function of JTX of the intestinal microbiota beneficially limits the post-surgical increases in the serum ammonia level. The mechanism by which JTX preserves individual specific intestinal microbiota pattern is not understood and needs further investigation.

Another benefit of JTX is that JTX, as opposed to the current therapies of orally administered lactulose and non-absorbable antibiotics, is that it did not influence bowel movements. Also JTX did not induce any hepatic or renal function disorders (Table 1). Thus, JTX can be administered safely to hepatic and renal function disorder patients. Clinically JTX is administered safely to patients with liver or renal dysfunction. 35,36 As a preventive therapy for hyperammonemia, administration of the traditional herbal medicine JTX may represent an important therapeutic advance for the modern surgical procedure of partial hepatectomy.

Conclusion

This study indicated that the oral administration of JTX suppressed the post-partial hepatectomy elevation of serum ammonia. The mechanism of action of JTX appears to be both stabilizing and inhibiting the surgically-induced change of intestinal microbiota. Further details of JTX’s pre-, pro- or anti-biotic activities should be further investigated.

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Japanese abstract

高アノミア血症に対する十全大補湯の効果と腸内菌叢の安定化作用を検討した。肝硬変患者における肝細胞腫の治療として外科的治療は最も優位が期待できる。しかし、術後合併症は治療に携わるものにとって重大な問題である。中でも高アノミア血症は肝性脳症の原因となる一方、残機能に影響を与え、術後の脳腫脹の低下により毎日肠管移動の誘因となる。

本研究ではマウスに十全大補湯を術前経口投与することで

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肝部分切除後の血清アンモニア値の上昇が抑制された。肝機能障害は十全大補湯では改善されなかった。十全大補湯非投与群では有意な血清アンモニア値の上昇を認めたが、投与群では上昇が抑えられた。T-RFLPによる腸内細菌の解析結果から肝部分切除術によりそのパターンの変化が認められたが、投与群ではパターンの変化は認められなかった。非投与群に対して、非手術群と十全大補湯投与群はCluster解析において72%の類似性を認めた。以上より、十全大補湯は肝部分切除後に認められる腸内細菌の変動を抑え、安定化させることによって血清アンモニア値の上昇を抑制しているものと考えられた。

*〒160-8582 東京都新宿区信濃町35
慶應義塾大学医学部漢方医学講座　渡辺賢治