# Effects of lactitol on intestinal microflora and plasma endotoxin in patients with chronic viral hepatitis

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**Summary** *Objectives:* To investigate the effects of lactitol on intestinal flora and the levels of plasma endotoxin in patients with chronic viral hepatitis.
*Methods:* Sixty patients with chronic viral hepatitis and gut-derived endotoxemia were randomly divided into two groups: lactitol group ($n = 30$) and control group ($n = 30$). Patients in the control group received standard medical treatment for 3 weeks, while patients in the lactitol group received lactitol orally in addition to the standard medical treatment. Fecal flora and plasma endotoxin were measured before and after the treatment.
*Results:* In the lactitol group, the numbers of *Bifidobacterium* and *Lactobacillus* per gram of wet feces were significantly increased ($p < 0.01$) and *Clostridium perfringens* count was decreased markedly ($p < 0.001$). The levels of plasma endotoxin decreased after the treatment from 72.89 ng/L to 33.33 ng/L in the lactitol group and from 66.00 ng/L to 51.07 ng/L in the control group, but the plasma endotoxin levels in the lactitol group decreased far more than in the control group ($p < 0.01$).
*Conclusions:* Lactitol can decrease the levels of plasma endotoxin more effectively than standard medical treatment in patients with chronic viral hepatitis through improving intestinal microflora.
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# Introduction

Various degrees of endotoxemia exist in patients with chronic viral hepatitis (CVH), and the presence of endotoxemia is associated with chronicity and severity of hepatitis.[1-3] The emergence of endotoxemia is closely correlated with disturbance of gut flora and the decline of colonization resistance (CR).[4] It is reported that the use of microecological preparations such as *Bifidobacterium* spp. and *Lactobacillus* spp. can decrease the plasma endotoxin by modulating intestinal flora in patients with chronic hepatitis (CH).[5,6] Lactitol is known as a prebiotic[7,8]; it can increase the quantity of the beneficial bacteria and decrease the population of putrefactive bacteria selectively, reduce the intestinal pH, and lower the production and absorption of ammonia.[9] Lactitol is widely used in treating encephalopathy and constipation,[10-12] but no reports are hitherto available on the treatment of chronic liver diseases with gut-derived endotoxemia. The current study was designed to investigate the effects of oral lactitol on intestinal flora and the levels of plasma endotoxin in patients with CVH.

# Patients and methods

Sixty patients with CVH included in the study were inpatients of the First Affiliated Hospital of Zhejiang University and the Fourth Hospital of Hangzhou, China. There were 53 males and 7 females, aged from 15 to 68 years, with an average of $33 \pm 16$ years. All patients were diagnosed as having CVH according to criteria set up by the Society of Infections and Parasitic Diseases of Chinese Medical Association in September 2000[13]; the levels of plasma endotoxin were equal to or higher than 45 ng/ml, no patients had taken drugs such as antibiotics, lactulose or living microbial food supplements in the 4 weeks prior to the study, and all maintained their usual dietary habits throughout the study. Fifty-eight cases were chronic hepatitis B, two cases were chronic hepatitis C. Patients with chronic cholecystitis, diabetes, Crohn's disease, ulcerative colitis or evidence of bacterial infection were excluded from the study.

Sixty patients were randomly divided into two groups: lactitol group ($n = 30$) and control group ($n = 30$), The control group was treated with standard medical treatment. The lactitol group patients took lactitol (Jiangsu Chia-tai Tianqing Pharmaceutical Co., Ltd.) orally after meals on the basis of standard medical treatment, three times daily; the lactitol dosage ranged from 15 g to 45 g/day to adjust the times of defecation to 2–3 times /daily; the treatment period was 3 weeks. The study was carried out according to the Guidelines of the Ethic Committee of the College of Medicine, Zhejiang University.

## Feces collection and bacterial identification

Freshly voided feces were collected in a sterile plastic bag from each person. One gram of stool was homogenized and diluted with anaerobic solution A (decimal up to $10^8$) in an anaerobic chamber (Forma Scientific Co., USA).

Determination of intestinal bacterial flora was performed with the modified methods described by Yoshikazu et al.[14] The specimens were cultured within 30 min after collection. Fifty microliters of serial dilutions ($10^{-1}, 10^{-3}, 10^{-5}, 10^{-7}, 10^{-8}$) were spread on the two non-selective agar media and seven selective agar media used previously.[3] The plates for the recovery of obligate anaerobes were incubated in an anaerobic chamber ($N_2:CO_2:H_2$ 8:1:1) at 37% for 48–72 h. The media used for the isolation of aerobes and facultative species were incubated in air for 48 h at 37%. After incubation, the bacterial identification was performed at family level (Enterobacteriaceae) or genus level (*Bacteroides*, *Bifidobacterium*, *Enterobacteriaceae*, *Enterococcus*, *Lactobacillus*, yeasts) or species level (*C. perfringens*) using standard bacteriologic techniques based on the morphology of the colonies on the plates, microscopic examination of Gram-stained slides, tests for growth under aerobic conditions, and various tests for biochemical characteristics.[15] Meanwhile, colony-forming units (CFU) per gram wet feces were calculated. In our study, the lowest detection limit was $2 \times 10^2$ organisms per gram of wet feces. The results were expressed as the $\log_{10}$ of the number of bacteria per gram weight of the wet fecal material.

## Plasma endotoxin levels

Plasma endotoxin was measured by means of modified chromogenic limulus amoebocytes assay (Shanghai Eihua Medical Co., Ltd).[16,17] Determinations of 45 ng/L or more were arbitrarily considered as positive.

## Statistical analysis

All the values were expressed as the mean $\pm$ SD, and Student's *t*-test was used to determine

**Table 1** Changes in intestinal microflora before treatment (BT) and after treatment (AT)

| Organism | Treated group[a] | | Control group[b] | |
|---|---|---|---|---|
| | BT | AT | BT | AT |
| *Bacteroides* | $10.32 \pm 0.78$[c] | $9.54 \pm 2.03$ | $10.43 \pm 1.15$ | $9.98 \pm 1.03$ |
| *Bifidobacterium* | $9.11 \pm 1.60$ | $10.04 \pm 1.26$[d] | $8.99 \pm 1.05$ | $9.13 \pm 1.58$ |
| *C. perfringens* | $7.85 \pm 1.80$ | $5.40 \pm 2.89$[e] | $7.40 \pm 1.39$ | $6.20 \pm 3.58$ |
| *Enterobacteriaceae* | $9.07 \pm 0.85$ | $8.49 \pm 1.85$ | $9.24 \pm 0.77$ | $9.44 \pm 1.05$ |
| *Enterococcus* | $8.33 \pm 1.15$ | $7.89 \pm 0.88$ | $8.44 \pm 1.58$ | $8.69 \pm 0.96$ |
| *Lactobacillus* | $4.18 \pm 3.13$ | $5.29 \pm 3.18$[d] | $4.25 \pm 3.21$ | $4.98 \pm 2.56$ |
| Yeasts | $2.14 \pm 2.47$ | $1.17 \pm 2.02$ | $2.91 \pm 2.36$ | $2.27 \pm 2.56$ |

[a] Group treated with lactitol in addition to routine treatment.
[b] Group given routine treatment.
[c] $Log_{10}$ bacterial CFU per gram of wet feces, mean $\pm$ SD.
[d] Results after treatment vs those before treatment for both groups, $p < 0.01$.
[e] Results after treatment vs those before treatment for both groups, $p < 0.001$.

significances. When $p$ was less than 0.05, the difference was considered statistically significant. Software SPSS10.0 was used in all statistical analyses.

## Results

### Changes in intestinal microflora before and after the treatment

As shown in Table 1, there was no significant change of intestinal microflora before treatment (BT) and after treatment (AT) in the control group. In contrast the intestinal microbes were changed significantly after lactitol administration, which resulted in a large increase in *Bifidobacteria* and *Lactobacillus* counts ($p < 0.01$), and a large decrease in *Clostridium perfringens* ($p < 0.001$). Furthermore, tendencies towards a decrease in *Enterobacteriaceae* and *Bacteroides* were observed, but with no statistical significance.

### Changes in plasma endotoxin levels in the control and lactitol groups before and after the treatment

In the control group there were 13 cases in which plasma endotoxin recovered to baseline after the treatment, whereas in four cases levels remained elevated. Overall, there was a significant reduction in endotoxin levels after treatment ($p < 0.05$) (Table 2). In the lactitol group there were 26 cases in which plasma endotoxin was restored to the normal range after treatment and no cases were found with elevated plasma endotoxin levels. Overall, there was a significant reduction in endotoxin levels following lactitol treatment

($p < 0.01$). Furthermore, as shown in Table 2, the decrease in endotoxin levels in the lactitol group was significantly greater than that in the control group ($p < 0.01$). Endotoxemia recovery rate was 86.6% in the lactitol group, higher than that of the control group (43.3%).

## Discussion

Gut-derived endotoxemia is often found in CVH patients.[1,18] It can exacerbate the injury of the liver and subsequently induce various complications. Many studies revealed that the subsequently changes such as the overgrowth of gram negative bacteria, the decline of intestinal bacterial CR and the lack of bile salt can enlarge the pool of endotoxin and increase the permeability of the intestinal mucosa in the intestine of CH

**Table 2** Changes in endotoxin in both groups before treatment (BT) and after treatment (AT)

| Group[a] | Case number | BT | AT |
|---|---|---|---|
| Treated group | 30 | $72.89 \pm 20.29$[b] | $33.33 \pm 15.63$[c,d] |
| Control group | 30 | $66.00 \pm 18.47$ | $51.07 \pm 23.17$[e] |

[a] Treated group was the patients who took lactitol in addition to the standard medical treatment. Control group was patients treated with standard medical treatment.
[b] Endotoxin, ng/mL $\pm$ SD.
[c] The treated group vs control group after treatment, $p < 0.01$.
[d] Results after treatment vs those before treatment for both groups, $p < 0.01$.
[e] Results after treatment vs those before treatment for both groups, $p < 0.05$.

patients.[3,19] Thus, the absorption of endotoxin from the intestine is increased, meanwhile the function of hepatic Kupffer cells is disturbed and the peripheral blood's capacity for endotoxin clearance is reduced in CVH, hence the induction of the gut-derived endotoxemia.[2]

Lactitol is a synthetic disaccharide of galactose and sorbitol. It has been widely used in the treatment of hepatic encephalopathy and constipation,[10] and is now known as prebiotic. Because a suitable galactosidase is lacking in the upper part of the gastrointestinal tract of humans, lactitol reaches the colon largely undigested.[8] Lactitol is used as an energy source by special intestinal microflora in the colon.[8,9,20] An in vitro study showed that Bifidobacterium and Lactobacillus can use lactitol more effectively than the other bacteria in the gut.[20] Tarao et al. treated eight patients with liver cirrhosis and hepatic encephalopathy with lactitol for 3—4 weeks to find a marked increase of Bifidobacterium and Lactobacillus, a decline in Bacteroides and Clostridium, increased frequency of defecation and improvement in mental state.[21]

No reports were found about the treatment of CVH with lactitol. Our previous studies revealed that disturbance of intestinal microflora was observed in patients with CVH; the counts of Bifidobacterium were significantly decreased, whereas the numbers of Enterococcus were increased.[3,18,22] We found in this study that the plasma endotoxin was decreased slightly under routine therapy in CH patients, but no significant change in intestinal microflora was found. The result indicated that with the recovery of the liver function, the hepatic Kupffer cells' capacity for endotoxin clearance was restored to some extent,[23,24] leading to the decrease of plasma endotoxin levels. In the lactitol group, beneficial intestinal bacteria such as Bifidobacterium and Lactobacillus were significantly increased in contrast to a marked decrease of C. perfringens with oral administration of lactitol. A significantly greater decline in endotoxin levels was found in the lactitol treated group than the control group. It is indicated that other factors can also take part in the recovery from gut-derived endotoxemia besides the enhancement of the hepatic ability to clear away the endotoxin. We postulate that the following reasons may participate in this phenomenon: (1) gut microbiological CR was increased after taking lactitol, shown by the great increase of Bifidobacterium and Lactobacillus. The Bifidobacterium can bind tightly to the epithelial cells of the intestinal mucous membrane via lipoteichoic acid, forming a bacterial membrane barrier with other beneficial

bacteria such as Lactobacillus, thus it leads to a reduction of absorption of endotoxin.[25—27] (2) Lactitol can improve the proliferation of lactic acid bacteria including Lactobacillus and Bifidobacterium, and the short fatty acids produced by the lactic acid bacteria can lower the intestine's pH;[9,28] therefore, it can also inhibit the growth of potentially pathogenic bacteria including C. perfringens, accelerate the movement of the intestine, and quicken the defecation of the intestinal endotoxin.[20,21,29]

Taking lactitol orally can not only regulate the intestinal microflora, but also reduce the levels of plasma endotoxin more effectively than routine treatment in CH patients. It can be used as an effective auxiliary method in the treatment of CVH, but more study is needed to establish and verify the effects of lactitol on intestinal microflora and endotoxemia in other types of the hepatitis.

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