• BASIC RESEARCH •

Effect of cisapride on intestinal bacterial and endotoxin translocation in cirrhosis

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Abstract

AIM: To investigate the effects of cisapride on intestinal bacterial overgrowth (IBO), bacterial and endotoxin translocation, intestinal transit and permeability in cirrhotic rats.

METHODS: All animals were assessed with variables including bacterial and endotoxin translocation, intestinal bacterial overgrowth, intestinal transit and permeability. Bacterial translocation (BT) was assessed by bacterial culture of MLN, liver and spleen, IBO by a jejunal bacterial count of the specific organism, intestinal permeability by determination of the 24-hour urinary ^{99m}Tc-DTPA excretion and intestinal transit by measurement of the distribution of ^{51}Cr in the intestine.

RESULTS: Bacterial translocation (BT) and IBO was found in 48 % and 80 % cirrhotic rats respectively and none in control rats. Urinary excretion of ^{99m}Tc-DTPA in cirrhotic rats with BT (22.2±7.8) was greater than these without BT (10.5±2.9). Intestinal transit (geometric center ratio) was significantly delayed in cirrhotic rats (0.31±0.06) and further more delayed in cirrhotic rats with BT (0.24±0.06) than these without BT (0.38±0.11). Cirrhotic rats with IBO had significantly higher rates of intestinal bacterial and endotoxin translocation, slower intestinal transit time and higher intestinal permeability than those without IBO. It was also found that BT was closely associated with IBO and the injury of intestinal barrier. Compared with the placebo group, cisapride-treated rats had lower rates of bacterial/endotoxin translocation and IBO, which was closely associated with increased intestinal transit and improved intestinal permeability by cisapride.

CONCLUSION: These results indicate that endotoxin and bacterial translocation in cirrhotic rats may be attributed to IBO and increased intestinal permeability. Cisapride that accelerates intestinal transit and improve intestinal permeability might be helpful in preventing intestinal bacterial and endotoxin translocation.

INTRODUCTION

Cirrhotic patients have an increasing susceptibility to bacterial infection, such as spontaneous bacterial peritonitis (SBP) and bacteremia, which are mainly caused by aerobic gram-negative organism of enteric origin[1,2]. Bacteria of enteric origin crossing the intestinal barrier to the mesenteric lymph nodes (MLN), a phenomenon known as bacterial translocation (BT) has recently been documented to occur commonly in cirrhotic rats compared to normal rats. BT has also been reported to be involved in the development of SBP in experimental models of ascitic cirrhosis[3-5]. The major mechanisms concerning bacterial translocation are deficiencies in local host immune defense, increased permeability of gut barrier and intestinal bacterial overgrowth (IBO). Certain pathological conditions such as shock, sepsis, trauma, burns, intestinal radiation, antibiotic overdose, malnutrition and immuno-suppression are closely related to BT and endotoxemia [6-8]. Although it has been showed that several mechanisms are involved in development of BT in liver cirrhosis[9-13], the increased intestinal permeability and IBO due to intestinal mucous membrane congestion and edema attributed to portal hypertension are considered the most important[13,14]. However, so far there have been not satisfied methods for prevention and treatment of intestinal endotoxemia. Strategies to reduce the intestinal bacterial translocation (BT) and endotoxemia in patients and experimental models of cirrhosis have mainly focused on the selective intestinal decontamination[15,16]. In this way the effectiveness of alternative antibiotics might be decreased with time because of the selection of resistant bacterial strains that could subsequently colonize the gut and become a potential source of infection, especially in patients with long-time prophylactic treatment. So nonantibiotic drugs are needed to be evaluated in the treatment and prevention of bacterial and endotoxin translocation in cirrhosis and decided whether or not to to be applied to the clinical practice[17].

Cisapride is a 5-HT$_4$ agonist that can accelerate the movement of the intestine. Many studies have reported that the intestinal bacterial and endotoxin translocation were closely related to IBO and intestinal hypomotility.

In this study we intend to study the effect of cisapride on intestinal transit and the permeability of gut barrier, two factors that are closely associated with intestinal bacterial and endotoxin translocation in cirrhotic rats.

MATERIALS AND METHODS

One hundred and sixty male Sprague-Dawley rats weighing 180-200 g were included in the study. Animals were caged in a controlled room temperature of 21 ℃ with a 12-hour light/dark cycle and fed standard rat diet with water *ad libitum*. The study was in accordance with guideline for animal research and was approved by the ethical and research committee of the hospital.

Cirrhotic animal model

Cirrhosis was induced in one hundred and thirty-five rats by subcutaneous injection of 50 % CCl4-olive oil solution twice

a week at an initial dose of 0.6 ml/100 g. Subsequent dosage was adjusted with body weight changes at a dose of 0.3 ml/100 g for 12 weeks. Seventy rats died during the induction of cirrhosis with a mortality of 50 % on average. At last sixty-five cirrhotic rats were used for further study.

Experimental design

25 rats were assigned as healthy controls (group 1). 65 cirrhotic rats were further divided into three groups. Group 2, which included 25 cirrhotic animals without any treatment, was used to study various parameter changes in cirrhosis. Group 3 was consisted of 20 cirrhotic animals with intragastric administration of cisapride suspension for two weeks and used to determine whether cisapride had effects on BT, endotoxemia, IBO, intestinal transit and intestinal permeability. Another 20 cirrhotic animals receiving equal volume of saline to cisapride suspension were named group 4 and used as cirrhotic controls.

Determination of parameters

Animals were fasted for 8 hours before killed. All experimental procedures were performed in sterile conditions. The animals were anesthetized by injection of 2 % pento-barbital natrium into abdominal cavity at a dose of 25-40 mg/kg. At the first day of experiment the rats were fed 5 μci of ^{99m}Tc-diethylenetriamine pentaacetic acid (^{99m}Tc-DTPA) (dissolved in 2 ml water) and housed individually in metabolic cages to collect 24-hour urine for further analysis. At the second day, after another 8-hour fasting animals were given 2 ml water containing 2 μci of ^{51}Cr through a gastric tube. Thirty minutes later animals were anesthetized and undergone a laparotomy under strict aseptic conditions. After small intestine was ligated at both ends MLN, liver, spleen and intestine were carefully removed out of the cavity. Blood samples were taken from the inferior vena cava.

Intestinal permeability

Intestinal permeability was determined by the 24-hour urinary excretion of ^{99m}Tc-DTPA. Results were expressed as fractional excretion of the radioactive substance. ^{99m}Tc-DTPA was a macromolecule and rarely absorbed into bloodstream through intestinal mucous membrane. When the intestinal permeability was increased as a result of intestinal mucous membrane injury, The absorption of DTPA into blood stream and thus excretion from urine would be increased. Therefore, increased excretion of DTPA from urine was assumed to be reliable index of intestinal permeability[18, 19].

Intestinal transit

Measurement of intestinal transit by determining the distribution of ^{51}Cr in the intestine was performed in all animals[20-22]. Special care was taken to prevent movement of intestinal contents in experimental procedures. After separated from the mesentery intestine was removed out of the abdominal cavity, put longitudinally in a moist container and then divided by the ligation of threads into 5-cm segments from orad to aborad. The radioactivity of every segment was measured with gamma-scintillation. Intestinal transit was expressed as the geometric center of ^{51}Cr distribution within the intestine and was calculated as the sum of the products of the fraction of the total administered dose of radioactivity per segment and the segment number. The geometric center were divided by the total number of segments of each rat to correct the difference in the length of intestine and finally expressed as geometric center ratio, which was regarded to be the most accurate method for measurement of intestinal transit.

BT studies

BT from the intestinal lumen was defined on the basis of positive culture of MLNs (particularly those draining lymph from ileum and cecum), liver, spleen and blood and excluding the infection from other possible sources. All the samples were immediately stored at -70 ℃ until detection. MLNs, liver and spleen were washed free of blood with sterile saline solutions (SS) and made 10 % tissue-slurry (1 g tissue plus 10 ml sterile SS), then immediately cultured in agar-blood medium plates.

IBO studies

IBO was defined as a jejunal bacterial count of the specific organism that was more than the mean plus two standard deviations of the same organism count in control rats. For the determination of IBO, 0.1 ml of jejunal contents were obtained under aseptic conditions by needle puncture. Then 20 μl of samples that were diluted 100 or 1 000 folds respectively were cultured in blood-agar plates. After an incubation period of 24-48 hours, the number of colony-forming units (CFUs) was counted. Moreover, the composition of the isolated flora was determined with standard identification techniques. The results were expressed as CFU/ml of jejunal contents.

Determination of serum endotoxin

All the blood specimens for the endotoxin determination were stored in endotoxin-free tubes. The serum was separated by 8 000 g 10 min. Serum level of endotoxin was determined by limulus ameobatic lysate (LAL) test with LAL kits (purchased from Shanghai medical-chemical institute).

Statistical analysis

Data are presented as means ±SD or proportions as required. Comparisons of quantitative variables among groups were made with the 1-way ANOVA or its corresponding nonparametric test as required. The χ^2 test was used for comparing proportion. The Spearman or Pearson test was used for correlation analyses when appropriate. A *P* value of <0.05 was considered statistically significant.

RESULTS

BT was found in 12 of 25(48 %) cirrhotic rats and none in control rats (Table 1, *P*<0.01). IBO was present in 20 of 25 cirrhotic rats (80 %) and none in the control rats. All the 12 cirrhotic rats with BT and 63 % of 13 cirrhotic rats without BT were found having IBO (Table 2). The translocated bacteria were Escherichia coli in 10 cirrhotic rats and Klebsiella P. and Enterococus in other two rats respectively. The same organism was always found at the same time both in BT and IBO. (Table 3).

BT was observed in 11 of the 20 rats with IBO and in only one of the five rats without IBO (55 % *vs* 20 %, *P*<0.05) (Figure 1). Endotoxin level was measured in the blood of inferior vena cava of all animals and higher endotoxin level was found in cirrhotic rats. Animals with BT or IBO have higher blood endotoxin level than that without. Intestinal transit was significantly delayed in cirrhotic rats and much more delayed in that with BT. This may result from IBO because cirrhotic rats with IBO have more delayed intestinal transit than that without.

Urinary ^{99m}Tc-DTPA excretion was greatly increased in cirrhotic rats than that of their controls. Although the urinary ^{99m}Tc-DTPA excretion in cirrhotic rats with IBO was more than that without the difference was not significant. Similarly urinary ^{99m}Tc-DTPA excretion in cirrhotic rats with BT was more than that without. All of these showed that severer impairment of mucous membrane barrier that had occurred in cirrhotic rats, which might be the key factor to promote occurrence of BT.

The mortality of rats was similar in both cisapride and placebo-treated animals. BT was present in 1 of the 20 cirrhotic rats treated with cisapride and in 11 of the 20 rats receiving placebo (5 % *vs* 58 %, *P*<0.01). IBO incidence in cirrhotic rats

receiving cisapride suspension was lower than that treated with placebo. Lower serum endotoxin level and faster intestinal transit was found in cirrhotic rats with cisapride treatment than that in the placebo group. (Table 4) Intestinal permeability as showed by the urinary [99mTc]-DTPA excretion was reduced significantly after cisapride treatment.

Table 1 Characteristics of control and cirrhotic rats

	Control rats	Cirrhotic rats
Number of animal	25	25
IBO(%)	0	20/25(80%)[b]
Total jejunal bacteria contents (CFU/ml)	0.54±0.18	1.59±0.48[b]
Bacterial translocation(%)	0	12/25(48%)[b]
Endotoxin level(pg/ml)	0.11±0.058	0.648±0.134[b]
Intestinal transit (geometric center ratio)	0.49±0.08	0.31±0.06[a]
Intestinal permeability (%urinary excretionof [99mTc]-DTPA)	1.62±0.8	16.1±7.6[b]

[b]$P<0.01$ *vs* control rats; [a]$P<0.05$ *vs* control rats.

Table 2 Characteristics of Cirrhotic rats with and without BT

	Cirrhotic rats with BT	Cirrhotic rats without BT
Number of animal	12	13
IBO(%)	100%	63%[a]
Total jejunal bacteria contents (CFU/ml)	2.61±0.56	0.65±0.12[b]
Endotoxin level(pg/ml)	0.873±0.137	0.440±0.108[b]
Intestinal transit (geometric center ratio)	0.24±0.06	0.38±0.11[a]
Intestinal permeability (%urinary excretion of [99mTc]-DTPA)	22.2±7.8	10.5±2.9[b]

[b]$P<0.01$ *vs* cirrhotic rats with BT; [a]$P<0.05$ *vs* cirrhotic rats with BT.

Table 3 Bacterial species cultured from mesenteric lymph nodes, liver, spleen, peripheral blood and overgrowth in the jejunum

No.	Mesenteric lymph nodes	Liver	Spleen	Blood	Intestinal bacterial overgrowth
1	E.coli	E.coli	E.coli	-	E.coli
2	E.coli	E.coli	E.coli	E.coli	E.coli
3	E.coli	E.coli	E.coli	E.coli	E.coli E.faecalis
4	E.coli	E.coli	E.coli	E.coli	E.coli
5	-	E.coli	-	-	E.coli
6	E.coli Ps. aeruginosa	E.coli	-	-	E.coli Ps. aeruginosa
7	E.coli	-	E.coli	-	E.coli
8	P klebsiella E.coli	-	-	-	P klebsiella E.coli
9	E.coli P. mirabilis	E.coli	-	-	E.coli P. mirabilis
10	-	E.coli	E.coli	-	E.coli
11	P enterococus E.coli	-	SP enterococus	-	P enterococus E.coli
12	E.coli	-	-	E.coli	E.coli

Abbreviations: E. coli, Escherichia coli; P. mirabilis, Proteus mirabilis; Ps. Aeruginosa, Pseudomonas aeruginosa, P klebsiella: Pneumonia Klebsiella.

Table 4 Effect of cisapride on BT, IBO, serum endotoxin level, intestinal transit and intestinal permeability of cirrhotic rats

	Placebo	Cisapride
Number of animal	20	20
Bacterial translocation (%)	11/20(55%)	2/20(10%)[b]
IBO(%)	16/20(80%)	3/20(15%)[b]
Total jejunal bacteria contents (CFU/ml)	1.60±0.42	0.60±0.58[a]
Endotoxin level(pg/ml)	0.721±0.123	0.148±0.079[b]
Intestinal transit (geometric center ratio)	0.33±0.08	0.68±0.16[b]
Intestinal permeability (%urinary excretion of [99mTc]-DTPA)	17.2±5.98	12.2±5.28[a]

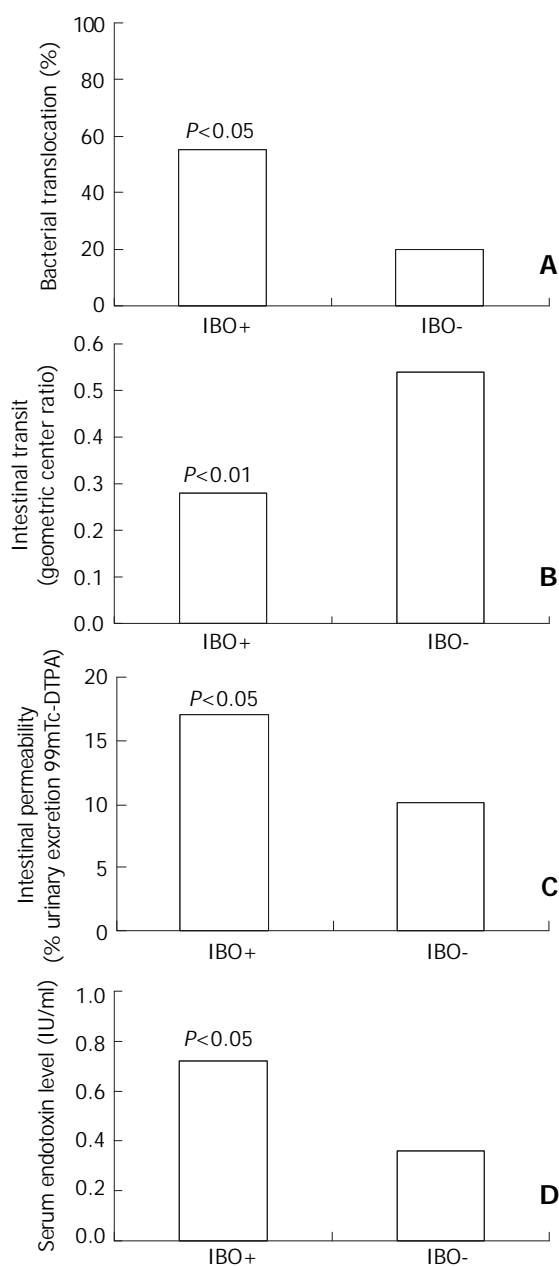[b]$P<0.01$ *vs* placebo; [a]$P<0.05$ *vs* placebo.

Figure 1 Bacterial translocation, intestinal transit, intestinal permeability and serum endotoxin level in cirrhotic rats with and without IBO (IBO+, IBO-, respectively). Cirrhotic rats with IBO have a higher incidence of BT (A), slower intestinal transit (B), higher intestinal permeability (C) and higher serum endotoxin level (D) than that of cirrhotic rats without IBO.

DISCUSSION

Many studies have shown a high susceptibility to bacterial infection among cirrhotic patients. In recent years, IBO and BT have been suggested to be involved in the pathogenesis of gut-origin bacterial infections, such as SBP, in animal with cirrhosis[21-23]. Although the phenomenon of BT has been recognized for more than a century, the precise mechanism of BT remains to be elucidated. IBO is postulated to be one of the major factors of BT. Guarner *et al*[24,25]. have shown that the intestinal aerobic bacterial count in cecal stool is significantly increased in CCl$_4$ induced cirrhotic rats with BT as compared with without and the prevalence of SBP was found to be significantly higher in cirrhotic patients with IBO than in those without. Reilly JA and Perez-Paramo M have reported that the incidence of IBO was significantly higher in cirrhotic patients with SBP than in those without[21,22]. Pardo *et al*[26] have observed that jejunal IBO were significantly higher in ascitic cirrhotic rats with BT than in those without, for a specific organism BT was always associated with its IBO, which suggests that the IBO favors the development of BT in experimental cirrhosis. In this experimental study, we have observed a direct relationship between the IBO and BT, which also suggests that IBO is one of the major mechanisms that promote BT in experimental models. However, The fact that not all cirrhotic rats with IBO developed BT suggested that other factors may play an important role in development of BT. It had been reported that impairment of gut barrier was a necessary process in the development of BT. Our result that higher value of urinary excretion of ^{99m}Tc-DTPA was seen in BT rats other than in that without was also suggested that the impairment of gut barrier was an important factor in promoting BT.

The mechanisms of gut barrier impairment were not completely elucidated. Some putative mechanisms have been proposed from animal and clinical studies. Portal hypertension in liver cirrhosis may be the most attractive factor in the impairment of gut barrier[26,27]. However, many studies have showed that poor linear relationship existed between the severity of high portal pressure and the impairment in intestinal permeability and that there was lack of improvement in permeability after reducing portal pressure[21]. It was possible that increased intra-luminal endotoxin level resulted from IBO played a contributory role in the damage to the gut barrier[21]. Our results of reduced incidence of IBO and improved intestinal permeability after cisapride treatment have shown the effect of endotoxin on the impairment of gut barrier.

Prevention of IBO was dependent on normal intestinal motility. Intestinal hypomotility was a main cause of IBO in cirrhotic animals[21,22,24,25]. This was supported by the delayed intestinal transit in cirrhotic animals with IBO and by the lower incidence of IBO in cirrhotic animals treated with cisapride, a drug that shortens bowel transit time.

During physiological processes, endotoxin is released from the bowel and detoxified by Kupffer cells and hepatocytes. High levels of endotoxin have been noted in cirrhotic patients. A number of previous studies have been shown that the plasma endotoxin level may be potentially helpful in the diagnosis of bacterial infection in patients with cirrhosis[27]. Recently a study revealed that increased levels of endotoxin indicated the occurrence of gram-negative bacterial infection[28,29]. In this study, we observed that the serum endotoxin level of the cirrhotic rats, especially those with IBO and BT, was much higher than that of healthy rats. The results suggested that endotoxemia caused by enteric bacteria was common in experimental cirrhosis and positively correlated with IBO and BT.

Several circumstances in cirrhosis could predispose a patient to IBO, such as alcohol abuse, malnutrition, hypochlorhydria, decreased intraluminal immunoglobin A or bile salts in the intestine, and disturbances of the small intestinal motility[6-8,12], Among which, prolonged intestinal transit, as a consequence of altered intestinal motility seems to play a major role in the development of IBO. Altered small intestinal motility was described in patients with cirrhosis[8,30,31]. Pardo and his associates[26] have recently found that alterations in small intestinal motility could result in a prolonged intestinal transit time in cirrhotic patients, which might facilitate the appearance of IBO and the 10-day treatment with prokinetic drug resulted in a marked reduction in jejunal bacterial content and BT in cirrhotic rats[21], which was coincide with our present study. In addition, and even more important, the prokinetic drug treatment was associated with a dramatic reduction in serum endotoxin level. Although the exact mechanisms by which the prokinetic drug reduce the incidence of BT, endotoxemia and IBO could not be completely elucidated on the basis of the present study, the observations that the serum endotoxin level was positively correlated with jejunal bacterial overgrowth, and that the prokinetic drug administration reduce not only IBO and BT but also endotoxin level, suggested that the beneficial effects of prokinetic drug may be due to the increasing of bowel movement and the promoting of intestinal bacterial and endotoxin elimination, which has been shown by shortened intestinal transit time in cisapride-treated group. Moreover, the administration of prokinetic drug could improve intestinal permeability in cirrhotic rats, which also suggested that increased intestinal permeability in cirrhotic rats was partially due to the damage of intestinal mucous membrane by bacteria overgrowth and high concentrations because cisapride has no direct protective effect on intestinal mucous membrane. In fact, it has been reported that prolonged the OCT could be significantly recovered in cirrhotic patients after cisapride therapy. These results suggested that the beneficial effect of the prokinetic drug on endotoxemia may be due to increasing the abolition of intestinal bacteria through the prokinetic effect. Unfortunately cisapride has lethal side-effect and has been prohibited to be used in treatment of disturbance of intestinal function in human. However, new prokinetic drugs have been available in the market and showed with similar effect on intestinal movement as cisapride. Therefore oral administration of prokinetic drugs might be beneficial to liver diseases by reducing absorption of endotoxin, a substance that is toxic to hepatocytes and could aggravate liver diseases.

In conclusion, the results of our experimental study indicated that the administration of prokinetic drug to cirrhotic rats resulted in a reduction of endotoxemia and BT incidence, which was companied by a marked decrease of IBO, reduced intestinal transit time and intestinal permeability. These findings suggested the beneficial effects of prokinetic drug on the prophylaxis of gut origin infection in cirrhosis, which shoud be taken as an adjuvant or alternative therapy to the selective intestinal decontamination with antibiotics.

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