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RAPID COMMUNICATION

Small intestinal bacteria overgrowth decreases small intestinal motility in the NASH rats

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Abstract

AIM: To explore the relationship between small intestinal motility and small intestinal bacteria overgrowth (SIBO) in Nonalcoholic steatohepatitis (NASH), and to investigate the effect of SIBO on the pathogenesis of NASH in rats. The effect of cidomycin in alleviating severity of NASH is also studied.

METHODS: Forty eight rats were randomly divided into NASH group (n = 16), cidomycin group (n = 16) and control group (n = 16). Then each group were subdivided into small intestinal motility group (n = 8), bacteria group (n = 8) respectively. A semi-solid colored marker was used for monitoring small intestinal transit. The proximal small intestine was harvested under sterile condition and processed for quantitation for aerobes (*E. coli*) and anaerobes (Lactobacilli). Liver pathologic score was calculated to qualify the severity of hepatitis. Serum ALT, AST levels were detected to evaluate the severity of hepatitis.

RESULTS: Small intestinal transit was inhibited in NASH group (P < 0.01). Rats treated with cidomycin had higher small intestine transit rate than rats in NASH group (P < 0.01). High fat diet resulted in quantitative alterations in the aerobes (*E. coli*) but not in the anoerobics (Lactobacill). There was an increase in the number of *E. coli* in the proximal small intestinal flora in NASH group than in control group (1.70 ± 0.12 log10 (CFU/g) *vs* 1.28 ± 0.07 log10 (CFU/g), P < 0.01). TNF-α concentration was significantly higher in NASH group than in control group (1.13 ± 0.15 mmol/L *vs* 0.57 ± 0.09 mmol/L, P < 0.01). TNF-α concentration was lower in cidomycin group than in NASH group (0.63 ± 0.09 mmol/L *vs* 1.13 ± 0.15 mmol/L, P < 0.01). Treatment with cidomycin showed its effect by significantly lowering serum ALT, AST and TNF-α levels of NASH rats.

CONCLUSION: SIBO may decrease small intestinal movement in NASH rats. SIBO may be an important pathogenesis of Nash. And treatment with cidomycin by mouth can alleviate the severity of NASH.

Key words: Nonalcoholic steatohepatitis; Small intestinal motility; Small intestinal bacteria overgrowth treatment

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INTRODUCTION

With much more prevalence of nonalcoholic fatty liver disease (NAFLD) than previously thought noticed, there has been an explosion on the research of pathogenesis of NAFLD. NAFLD may develop into cirrhosis of liver through NASH, in some cases, to hepatocellular carcinoma[1-5], Insulin resistance and peroxidative injury were thought to place an important role in the pathogenesis of NASH[6-12]. But there are still other mechanisms that can trigger and maintain NASH[13-18]. Small intestinal bacterial overgrowth has been reported to place a role in the pathogenesis of NASH, endotoxin and TNF-α being the possible mediator[19-21]. But contrary to this hypothesis, in another study, antibiotic treatment did not normalize aminotransferase levels in NASH patients[22]. Therefore more study is needed on the influence of SIBO on the pathogenesis of NASH and the curative effect of antibiotic on NASH. Alejandro S[22] found that prolonged Orocecal Transit Time (OCTT) in NAFLD patients coexist with SIBO. It is generally thought that prolonged OCTT is the cause of SIBO. But it is not clear if alteration of intestinal motility is a cause or a consequence of SIBO.

We established an NASH animal model by high fat diet to explore the relationship between intestinal motility and SIBO in NASH. The relationship between SIBO and NASH, and the curative effect of antibiotic on NASH were also studied.

MATERIALS AND METHODS

Materials

Forty eight male SD strain rats were used in this study.

Rats were housed individually in cages at constant room temperature in a 12-h light/dark cycle and had free access to laboratory feed and water. Semi-solid colored marker (carbon-ink 85%, gum acacia 10%, activated charcoal 5%) was made in our laboratory. Bacteria evaluation kit (API 20) was purchased from French Biomerieux CO. TNF-α kit was purchased from Technology Development Center of the General Hospital of PLA.

Establishment of animal mode

Forty eight SD rats were randomly divided into NASH group, cidomycin group and control group. Each group were subdivided into small intestinal motility group (n = 8) and bacteria group (n = 8) respectively. Rats in NASH group and cidomycin group were fed with high fat diet that was made by ordinary diet (88%) plus fat (10%) and cholesterol (2%) (supported by Qinglongshan Nursery) Rats in control group were fed with ordinary diet (supported by Qinglongshan Nursery). Furthermore, rats in cidomycin group were treated with cidomycin (12Mu ig qd) after eight weeks of high fat diet while those in the other two groups were treated with isotonic Na chloride (1 mL/d ig qd). At the end of the twelfth week, when the rats in NASH group and cidomycin group had received high fat diet for 12 wk, all the rats were killed, the serum levels of aminotransferase, TNF-α were tested and the histology of liver specimen was observed by H&E staining.

Measurement of small intestinal transit

Rats in small intestinal motility group was deprived of food for 24 h and water for 12 h prior to measurement of small intestinal transit. Then 1.0 mL semi-solid colored marker was administered into stomach by orogastric gavage. Twenty minutes later, the rat was killed, abdomen was opened and small intestine was dissected. The distance traveled by the marker was calculated. The small intestinal transit was represented by ratio of the distance traveled by the marker to the total length of the small intestine.

Histological evaluation

The liver pathologic score was calculated according to the lecture[23]: Inflammation in portal canal area was denoted by "P", inflammation inside lobules of liver by "L", piecemeal necrosis by "PN" and bridging necrosis by "BN". According to the severity of inflammation and necrosis, every item was scored from one to four. The total score of the liver inflammation was "P+L+2PN+2BN".

Measurement of small intestinal bacteria

Rats in the bacteria group was deprived of food for 24 h and water for 12 h prior to measurement of small intestinal bacteria. Then the rat was killed, the abdomen was opened and the proximal small intestine was harvested under sterile condition. A two-centimeter-long small intestine was dissected from the point about ten centimeters from pylorus, then rinsed with sterile saline thrice, and next, the leftover was sucked by sterile filter paper. After weighing, the leftover and 2 mL sterile saline were placed in a sterile glass homogenizer and homogenized. The homogenate was diluted with sterile saline at the ratio of 1:1, then, 100 μL dilution was plated on SS agar (*E. coli*) and MRS agar (Lactobacilli) respectively. The quantity of *E. coli* was determined after 24 h of aerobic cultivation at 37℃. While the quantity of Lactobacilli was determined after 48 h of anaerobic cultivation at 37℃. The number of Colony forming units (CFU) of bacteria was quantified. The quality of aseptic manipulation was evaluated by inoculating swab of abdominal cavity on sheep-blood agar. The serum TNF-α levels were detected, using radio-immunity method, in our laboratory.

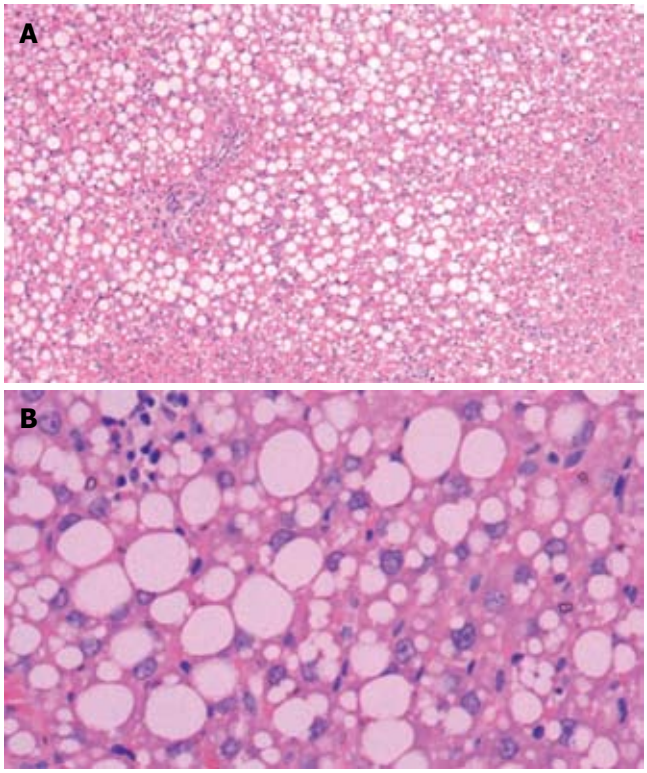

Figure 1 Pathological changes of liver after 12 wk of high fat diet. **A**: Fat drops in more than one thirds of liver cells (HE, × 40); **B**: Lymphocytes in hepatic steatosis background (HE, × 100).

Statistical analysis

Throughout this report, data were expressed as mean ± SD. Experimental results were analyzed by one-factor analysis of variance. $P < 0.05$ was considered statistically significant.

RESULTS

The weight of the model group rise *vs* controls was 455.38 ± 11.48g *vs* 395.38 ± 10.91 g, $P < 0.05$.

Pathologically a NASH model has been successfully made (Figure 1).

TNF-α, liver pathologic score, serum ALT and AST levels are higher in the NASH group than in the other two groups indicating a NASH model has been successfully made ($P < 0.01$, $P < 0.01$, $P < 0.05$). There are no statistical differences between the control group and the cidomycin group in serum TNF-α level. Liver pathologic score, serum ALT and AST levels are higher in cidomycin group than in control group ($P < 0.01$) (Table 1).

Table 1 The concentration of TNF-α, liver pathologic score, serum ALT and AST levels (mean ± SD)

	n	TNF-α (ng/mL)	ALT (U/L)	AST (U/L)	Liver pathologic score
Control group	8	0.57 ± 0.09[b]	39.73 ± 5.10	168.00 ± 16.41	1.00 ± 0.93
NASH group	8	1.13 ± 0.15	86.63 ± 20.91[d]	379.63 ± 61.73[d]	6.00 ± 1.20[d]
Cidomycin group	8	0.63 ± 0.09[b]	70.88 ± 11.93[a,d]	318.75 ± 52.62[a,d]	4.25 ± 1.58[a,d]

[b]$P < 0.01$ *vs* NASH group; [d]$P < 0.01$ *vs* control group; [a]$P < 0.05$ *vs* NASH group.

Table 2 Small intestinal bacteria, small intestinal transit rate (mean ± SD)

	n	*E. coli* [$\log_{10}$(CFU/g)]	*Lactobacilli* [$\log_{10}$(CFU/g)]	Small intestinal motility(fraction)
Control group	8	1.28 ± 0.07	1.67 ± 0.16	0.58 ± 0.06
NASH group	8	1.70 ± 0.12[b]	1.69 ± 0.16	0.39 ± 0.11[b]
Cidomycin group	8	1.17 ± 0.08[a,d]	1.81 ± 0.13	0.51 ± 0.07[d]

[b]$P < 0.01$ *vs* control group; [a]$P < 0.05$ *vs* control group; [d]$P < 0.01$ *vs* NASH group.

The number of *E. coli* in the proximal small intestinal flora are more in the NASH group than in the control group and cidomycin group ($P < 0.01$, $P < 0.01$). Small intestinal motility are weaker in NASH group than in control group and cidomycin group ($P < 0.01$, $P < 0.01$). The number of *E. coli* are less in cidomycin group than in control group ($P < 0.05$). There are no statistic difference in small intestinal motility between control group and cidomycin group. There are no statistical differences in the number of Lactobacilli between every two groups of the three (Table 2).

DISCUSSION

It was found that NASH coexist with SIBO[19,24]. In our study excessive multiplication of *E. coli* and increased serum level of aminopherase coexist in NASH rats, that was consistent with previous studies. We thought that SIBO is an important, though not the only, pathogenesis of NASH by the fact that antibacterial treatment can alleviate the severity of NASH. We also found the level of aminopherase went up and down with serum level of TNF-α. It strongly supported TNF-α to be an important mediator in the promotion of NASH by SIBO. In our study, rats treated with cidomycin had a serum level of aminopherase slightly higher than controls and a serum level of TNF-α similar to controls. The reason or this phenomenon might be that TNF-α is not the only mediator. Generally endotoxemia was thought to be a link between SIBO and elevated TNF-α level[19,20]. But Wigg A J[25] observed that serum levels of TNF-α and endotoxin were not parallel in NASH patients. Given the disadvantage of clinical trials to control experimental conditions, and the disadvantage of C^{14} breath test in identification of bacteria, animal experiments are necessary to find the link between SIBO and NASH. Our study found a significant increase in the number of *E. coli*, a widely known source of endotoxin, in the proximal small intestinal flora in NASH group. It might support endotoxin to be the promoter to TNF-α in NASH.

With respect to the causality between SIBO and small intestinal motility in NASH, ALEJANDRO-S[22] found that delayed intestinal transit coexist with SIBO in patients effected by NASH. We also observed that NASH rats suffered both delayed intestinal motility and an increase in intestinal E. coli. It is widely believed that delayed intestinal motility could cause SIBO. Leveau P *et al*[26] noticed a delay in intestinal transit time, appeared as an early event in acute pancreatitis, preceding intestinal bacterial overgrowth, and suggested that impairment in intestinal motility probably played a role in the development of bacterial overgrowth. Gangarosa[27] demonstrated that intestinal motility served as a normal cleansing mechanism of the intestine, and drugs that decreased this motility might facilitate replication of pathogens and their attachment to or invasion of the intestinal tissue. Nieuwenhuijs *et al*[28,29], clarified the role of the migrating motor complex (MMC) in the regulation of small intestinal microflora by the fact that the disruption of MMC with morphine promotes duodenal bacterial overgrowth. Since MMC can also coordinate with the movement of the pylorus, gut and cholecyst, the alteration of MMC necessarily has an influence on the secretion of bile (the inhibiter of gram-negative bacilli). MMC might influence intestinal bacteria *via* another mechanism. Grzesiuk *et al*[30], demonstrated that intestinal bacteria, particularly those adhering to intestinal epithelial cells, were exposed to electric fields and currents generated by the muscular activity of the small intestine and that the myoelectrical activity of the duodenum, through action on cell membrane, can affect cell division of intestinal bacteria.

Though it is generally believed that insulin resistance can explain the delay of small intestinal motility in NASH, and abnormality of intestinal bacteria may be caused by delayed intestinal motility, in our experiment, treatment with antibiotics improved the abnormality of small intestinal motility in NASH rats, indicating that small intestinal bacterial can also regulate intestinal motility in NASH rats. The interpretation may be founded on the influence microflora has on intestinal smooth muscle cell myoelectricity action. Huseby *et al*[31] demonstrated that after introduction of conventional intestinal microflora the interval between activity fronts of the MMC in proximal jejunum of germ-free rats was reduced. Sjogren *et al*[32] reported that bacterial adherence to the intestinal mucosa appeared to be important in eliciting the abnormal myoelectric responses. Different intestinal bacterial genus may have different influence on intestinal motility. *E. coli* adherence to intestinal mucosa delayed spike bursts[29], reduced the frequency and amplitude of the intestinal contractions and inhibit small intestinal transit[29]. while

Lactobacillus promoted regular spike burst activity, reduced the MMC period and accelerated small intestinal transit[29]. In our study the overgrowth of *E. coli* coexist with the reduction of small intestinal motility, and the elimination of *E. coli* could increase the small intestinal motility, strongly supporting *E. coli* to be an important mediator in the small intestinal motility in NASH rats. In our study Lactobacillus didn't change according to high fat diet or cidomycin treatment, indicating Lactobacillus may not be important in the pathogenesis of NASH.

The results of this study suggest that SIBO may be not only the result of but also a promoter to delayed intestinal motility in NASH. There might be a vicious circle between SIBO and delayed intestinal motility in NASH rats. SIBO can trigger NASH *via* TNF-α, and treatment with cidomycin orally can alleviate the severity of NASH.

COMMENTS

Background

While once considered a benign process, nonalcoholic steatohepatitis (NASH) has been found to lead to cirrhosis and, in some cases, to hepatocellular carcinoma. The mechanisms underlying disease development and progression are awaiting clarification; however the finding that not all patients with steatosis develop hepatic inflammation and hepatocellular damage has led to the hypothesis that different pathogenic factors lead firstly to hepatic steatosis and secondly to hepatic damage ("the second hit"). Insulin resistance and obesity-related inflammation, among other possible genetic, together with oxidative stress, microcirculation disturbance, malnutrition are thought to play a key role in the second stage. People also found that small intestinal bacteria overgrowth (SIBO) may also play a role in the process of NASH. Some people think the medium of TNF-α to be an endotoxin, but all people do not accept this.

Research frontiers

Great effort has been and is still being done to clarify all the possible mechanism in the development of NASH, The source and pathogenesis of mediators of inflammation in the process has been the hotspot. People also want to know if it can act as a target point in the treatment of NASH.

Innovations and breakthroughs

In this article the relationship between the small bowel moment and SIBO is studied. And the cidomycin was first studied for the treatment of NASH.

Applications

This article helps to understand the process of SIBO leading to NASH, and suggests that Cidomycin might be useful in the treatment of NASH. Since there might be a vicious circle between SIBO and delayed intestinal motility, the treatment of accelerator of intestinal motility might also be useful.

Terminology

NASH: A liver disease, which resembles alcoholic liver disease consists of steatosis plus inflammation, necrosis, and fibrosis while lack of history of drinking. SIBO: Small intestinal bacterial overgrowth.

Peer review

This article tries to study the relationship between SIBO and delayed intestinal motility in NASH rats and suggest that cidomycin might be effective in the treatment of NASH. But the conclusion needs further probation in future clinical study before clinical application.

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