**Effect of lactulose on establishment of a rat non-alcoholic steatohepatitis model**

Jian-Gao Fan, Zheng-Jie Xu, Guo-Liang Wang

**Abstract**

AIM: To explore the relationship between changes of intestinal environment and pathogenesis of non-alcoholic steatohepatitis (NASH).

METHODS: Forty-two Sprague-Dawley rats were randomly divided into model group (n = 24), treatment group (n = 12), and control group (n = 6). The rats of model and treatment groups were given high-fat diet, and those of the control group were given normal diet. Furthermore, the rats of treatment group were given lactulose after 8 wk of high-fat diet. Twelve rats of the model group were killed at 8 wk of high-fat diet. At the 16 wk the rats of treatment group, control group, and the rest of the model group were killed. The serum levels of aminotransferase were measured and the histology of livers was observed by H&E staining.

RESULTS: The livers of rats presented the pathological features of steatohepatitis with higher serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in the model group after 16 wk. Compared to the model group, the serum levels of ALT and AST in treatment group decreased significantly and were close to the normal group, and the hepatic inflammation scores also decreased markedly than those in the model group after 16 wk (5.83±0.64 vs 3.63±0.64, P<0.05), but were still higher than those in the model group after 8 wk (3.63±0.64 vs 1.98±0.90, P<0.05). However, the degree of hepatic steatosis had no changes in treatment group compared to the model group after 16 wk.

CONCLUSION: Lactulose could ameliorate the hepatic inflammation of rats with steatohepatitis induced by fat-rich diet, but could not completely prevent the development of steatohepatitis. It is suggested that intestinal environmental changes such as intestinal bacterial overgrowth, are one of the important factors in the pathogenesis of NASH.

**Key words:** NASH; Lactulose; Intestinal environment

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anesthetized with 1% pentobarbital by intraperitoneal injection. Then the blood samples were collected through abdominal aorta, the liver tissues were weighed and fixed in 40 g/L formaldehyde, embedded in paraffin.

**Biochemical measurement**

The serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglycerides (TG), and total cholesterol (TC) were measured using an automatic biochemical analytical system.

**Serum endotoxin level**

Serum endotoxin level was measured by chromogenic limulus amoebocyte lysate test in Shanghai Clinical Test Center, and the tubes without pyrogen were supplied by the center.

**Hepatic histology**

The liver tissue sections were stained with hematoxylin and eosin. Each section was assessed under 10×20 light microscopic fields and scored for the severity of steatosis and inflammation according to the following criteria: Steatosis was scored as: grade 0: no fat; grade 1: fatty hepatocytes occupying less than 33% of the hepatic parenchyma; grade 2: fatty hepatocytes occupying 34-66% of the hepatic parenchyma; and grade 3: fatty hepatocytes occupying more than 66% of the hepatic parenchyma. The diagnosis of fatty liver could be confirmed, when fatty hepatocytes occupying more than 33% of the hepatic parenchyma. Portal inflammation (P), intralobular inflammation (I), piecemeal necrosis (PN), and bridging hepatic necrosis (BN) had a score from 1 to 4 according to the pathologic severity. PN and BN had a greater correlation with the prognosis, the score was two times higher than P and L. The inflammation score was P+L+2PN+2BN.

**Statistical analysis**

All results were expressed as mean±SD. Statistical differences between means were determined by Student’s t test. Rank sum test was used in enumeration of data. P<0.05 was considered statistically significant.

### RESULTS

During the experiment, no rat died in three groups. In the treatment group, there were no marked increase in the volume of feces. The feces were soft. No significant difference was found in the body weights between model group and control group. But the ratio of the liver wet weight with the body weight in the model group increased significantly than that in the control group. In the treatment group, the body weights were lower than those in the model group at wk 16, and the ratio of the liver wet weight with the body weight (liver exponent, %) significantly decreased compared to that in the model group at wk 8 and 16 (Table 1).

#### Serum lipid

In model group, the serum level of TC was markedly higher than that in control group, but the serum level of TG was similar to that in control group. No significant difference in serum levels of TC between the model group and the treatment group, but the serum level of TG in the treatment group decreased markedly (Table 2).

#### Serum aminotransferase

The serum levels of ALT and AST in the model group had an increasing tendency at wk 8, but no significant difference was found between the model group and control group until wk 16. The serum levels of ALT and AST in treatment group decreased significantly, and almost became normal (Table 3).

#### Serum endotoxin level

The serum endotoxin level in portal vein was higher than that in abdominal aorta, both in model group and control group (P<0.05), but there was no significant difference in serum endotoxin level both in portal vein and in abdominal aorta between the two groups. Therefore, we did not measure the serum endotoxin level in treatment group (Table 4).

#### Hepatic histology

The livers of control group had normal morphology, while

### Table 1 Rat body weight and the liver exponent (mean±SD)

| Group     | Time (wk) | n  | Body weight (g) | Liver exponent (%) |
|-----------|-----------|----|-----------------|--------------------|
| Control   | 16        | 6  | 478.2±36.8      | 2.27±0.28          |
| Model     | 8         | 12 | 425.8±26.7      | 3.37±0.05*         |
|           | 16        | 12 | 505.8±60.1      | 3.93±0.51*         |
| Treatment | 16        | 12 | 433.3±58.2      | 2.96±0.24*         |

*P<0.05 vs model group; *P<0.01 vs control group.

### Table 2 Serum lipid changes (mean±SD)

| Group     | Time (wk) | n  | TG (mmol/L) | TC (mmol/L) |
|-----------|-----------|----|-------------|-------------|
| Control   | 16        | 6  | 0.83±0.20   | 1.10±0.18   |
| Model     | 8         | 12 | 0.68±0.18   | 1.81±0.30*  |
|           | 16        | 12 | 0.83±0.22   | 2.00±0.38*  |
| Treatment | 16        | 12 | 0.44±0.14   | 2.06±0.32   |

*P<0.05 vs model group; *P<0.01 vs control group.
the livers of model group and treatment group became yellow, dull, enlarged, fragile, and full. Microscopically, the livers of control group had no marked abnormality. At wk 8, the livers of model group were engorged with microvesicular and macrovesicular fat. Fatty liver could be diagnosed in 11 of 12 rats. Four out of twelve rats had mild intralobular inflammation. At wk 16, hepatic steatosis was much severe, and intralobular areas were infiltrated by mixed inflammatory cells in model group. These lesions were located mainly in zone 3 areas. Intralobular inflammation was more severe than portal inflammation. Some livers had several large areas of necrosis melted by focal intralobular inflammation. Two rats had hepatic piecemeal necrosis and three had bridging necrosis. In treatment group, the degree of hepatic steatosis slightly decreased, while the score of hepatic inflammation activity significantly decreased compared to those in model group. No hepatic piecemeal necrosis and bridging necrosis were found in treatment group (Tables 5 and 6).

**DISCUSSION**

The histological characteristics of NASH resemble those of alcoholic steatohepatitis, suggesting that both diseases may have a similar pathogenesis and can benefit from similar therapies\[15-18\]. Studies in alcohol-fed rodents have shown that intestinal bacteria, bacterial endotoxin, and endotoxin-inducible cytokine can modulate alcohol-induced liver damages including hepatic necrosis and fibrosis\[19,20\]. Treatment with antibiotics and lactobacillus that inhibit production of endotoxin by the intestinal flora can significantly inhibit the development of steatohepatitis in alcohol-fed animals\[21,22\]. Although the intestinal flora is known to play a critical role in the pathogenesis of alcohol-related liver disease, its role in NASH has been poorly understood. Surgical procedures (such as jejuno-ileal bypass) and TPN cause intestinal stasis and secondary bacterial overgrowth, accelerate the progression of fatty liver disease in obesity patients, suggesting that increased exposure to intestinal bacterial products may contribute to the pathogenesis of NASH\[1-5\]. It has been reported that the prevalence of small intestinal bacterial overgrowth is high in patients with NASH\[15\]. Lactulose could be fermented by colonic bacteria and turn into lactic acid and acetic acid, which can lower colonic

| Table 3 Serum aminotransferase changes (mean±SD) |
|-----------------------------------------------|
| Group     | Time (wk) | n  | ALT (U/L) | AST (U/L) |
| Control   | 16        | 6  | 34.16±5.17| 144.00±21.59|
| Model     | 8         | 12 | 64.54±40.54| 224.45±54.07 |
| Treatment | 16        | 12 | 47.8±11.0 | 133.7±26.5 |

\*P<0.05, \*P<0.01 vs control group; \*P<0.05, \*P<0.01 vs model group.

| Table 4 Rat blood endotoxin level (mean±SD) |
|---------------------------------------------|
| Group     | Time (wk) | n  | Serum endotoxin level (EU/mL) |
|           |           |    | Portal vein   | Abdominal aorta |
| Control   | 16        | 6  | 0.291±0.08     | 0.125±0.03     |
| Model     | 8         | 12 | 0.264±0.07     | 0.089±0.02     |
| Treatment | 16        | 12 | -              | -              |

| Table 5 Degree of hepatic steatosis in different groups |
|--------------------------------------------------------|
| Group     | Time (wk) | n  | Degree of steatosis |
|           |           |    | -   | +   | ++  | +++ |
| Control   | 16        | 6  | 6   | 0   | 0   | 0   |
| Model     | 8         | 12 | 0   | 1   | 6   | 5   |
| Treatment | 16        | 12 | 0   | 0   | 1   | 11  |

| Table 6 Score of rat hepatic inflammation activity (mean±SD) |
|------------------------------------------------------------|
| Group     | Time (wk) | n  | Score of inflammation activity |
| Control   | 16        | 6  | 0.66±0.76 |
| Model     | 8         | 12 | 1.98±0.90 |
| Treatment | 16        | 12 | 3.63±0.64 |

\*P<0.05 vs model group; \*P<0.01 vs control group.
pH, diminish ammonia production and normalize intestinal transit; therefore, lactulose syrup can be used in treatment of hepatic encephalopathy. Lactulose promotes the growth of acidophilous lactobacilli, bifidobacteria, and Gram-positive bacteria, while inhibits Gram-negative bacteria and prevents gut-derived endotoxemia. In order to explore the relationship between the change of intestinal environmental and pathogenesis of NASH, we observed the effect of lactulose on NASH rats.

The treatment group was given lactulose after 8 wk of high-fat diet, when simple fatty liver developed in rats. The model group developed NASH after 16 wk, while treatment with lactulose for 8 wk improved both serum aminotransferase and hepatic inflammation. These results suggest that intestinal environmental changes, such as small intestinal bacterial overgrowth, increased intestinal permeability and subsequent gut-derived endotoxemia may play an important role in the development of NASH. Although the treatment group received lactulose for 8 wk, the hepatic inflammation scores were still higher than those in model group, indicating that lactulose can ameliorate hepatic inflammation, but cannot prevent NASH. Lactulose could not improve hepatic steatosis in treatment group, suggesting that the change of intestinal environment is not closely related to hepatic steatosis.

In our serial researches, we found that the serum endotoxin level in NASH rats were significantly elevated, when hepatic fibrosis occurred after 24 wk of high-fat diet. The serum endotoxin level in portal vein and peripheral vessels had no significant difference between model group and control group, but the serum endotoxin level in portal vein was markedly higher than that in peripheral vessels in both groups. These results suggest that SD rats might have mild endotoxia. We also found that the expression of endotoxin receptors-CD14 and toll-like receptor 4 was upregulated in model group, suggesting that Kupffer cell sensitivity to endotoxin increases and low doses of endotoxin might injure liver.

Oral administration of lactulose may improve hepatic inflammation of NASH rats by reducing serum endotoxin level in portal vein.

Furthermore, other bacterial products such as peptidoglycan-polysaccharide polymers rather than endotoxin, could stimulate Kupffer cells and injure liver, because bacterial species rather than aerobic Gram-negative bacteria such as Escherichia coli may play a role in the pathogenesis of small intestinal bacterial overgrowth.

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