FXR mediated bile acid signal to advance the study of cirrhosis of the liver regeneration

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ABSTRACT

To explore the mechanism of FXR mediated bile acid signal to cirrhosis of the liver regeneration. The total sample size of 75 male SD rats were randomly divided equally into 3 groups ie, 25 samples in each group. The three groups were cholic acid deficiency group, cholic acid load group and control group. Cholic acid load group of forage feed 0.2% cholic acid, cholic acid deficiency group feeding to enamine with 2%, the control to the fed standard feed. In all the groups continuous feeding was done for 1 week. Serum and residual liver specimens of rats were collected. Liver wet weight and bile acid levels before and after removal were also recorded. Liver body ratio, AST and ALT, HGF levels, liver specimens, protein levels of Nester derivative receptor (FXR) and its target genes CYP7A1 and FOXM1B were detected by various laboratories investigations. The hepatosomatic ratio of all the three groups increased with time, but the hepatosomatic ratio of the cholic acid deficiency group was lower than that of the cholic acid load group and the control group (P < 0.05). There were no significant differences in ALT, AST and HGF levels among the three groups at the end of surgery (P > 0.05), but from the 3rd day after surgery, ALT and AST levels in the cholic acid deficient group were higher than those in the cholic acid-loaded group and the control group, while HGF levels were lower than those in the cholic acid-loaded group and the control group (P < 0.05). On the 7th day after surgery, the expression of FXR target genes CYP7A1 and FOXM1B increased, and the content of FOXM1B in the cholic acid deficiency group was the highest, up to 2.33±0.36, which was statistically significant compared with the control group and the cholic acid loaded group (P < 0.05), while the expression of FXR was the lowest in the cholic acid deficiency group (P < 0.05). The maintenance of normal intestinal bile acid content plays a key role in FXR mediated bile acid signaling to promote the regeneration of sclerosing liver, which provides a certain basis for the clinical application of cholic acid in the perioperative period of partial hepatectomy of sclerosing liver.

Keywords: Bile acid signal; Cirrhosis of the liver. Method the ester derivatives receptor; CYP7A1
INTRODUCTION
Primary liver cancer is a common malignant tumor in China, and its morbidity and mortality rank high among cancers. However, more than 80% of patients with liver cancer in China are complicated with cirrhosis. After hepatectomy, liver regeneration ability is significantly reduced, and improving the compensatory ability of residual liver is an urgent problem to be solved. 1, 2 Farnesoid X receptor (FXR) dependent bile acid signaling is essential for normal liver regeneration. 3 However, there are few reports on whether reduced bile secretion and endotoxemia in sclerosing liver also influence liver regeneration through nuclear receptor-dependent bile acid signaling and the extent of the influence. Therefore, in this study, the expression of FXR, CYP7A1 and FOXM1B as well as liver regeneration and liver function indexes ALT and AST were observed by changing the levels of cholic acid in cirrhosis rats to determine the role of nuclear receptor-dependent bile acid signaling in regulating residual liver regeneration after partial hepatectomy of sclerosing liver. To provide the basis for clinical application of cholic acid in perioperative period of partial hepatectomy of sclerosing liver.

1. Materials and Methods
1.1 Experimental animals and grouping
A total of 75 clean male adult rats, weighing 200-270g, were purchased from Guangzhou Leidewenxuan Biotechnology Co., Ltd., and raised in the Physiological Experimental Animal Center of Guangzhou Medical University. The feeding environment was kept at 23-25°C, relative humidity at 60-70%, artificial light and shade for 12 hours each, and 5 rats were placed in each cage. Standard feed and tap water were given for 1 week. 25 SD rats were divided into 3 groups randomly.

The three groups were cholic acid deficiency group, cholic acid load group and control group. Cholic acid load group of forage feed 0.2% cholic acid, cholic acid deficiency group feeding to examine with 2% of the test, the control group was feeding to standard feed and further continuous feeding 1 week after 55% of cirrhosis liver resection.

1.2 Experimental method
1.2.1 Establishment of rat liver cirrhosis regeneration animal model
The cirrhosis model of rats was established by referring to the cirrhosis model method established by Bin Wenting et al. 4, 5, that is, injecting 50% CCl4 olive oil solution + ethanol solution (prepared with Erguotou liquor and distilled water) as drinking water. The dosage of choleenamine and cholic acid was set by referring to the drug instructions of choleenamine and cholic acid and the dosage of related literature. The dosage used in this experiment was 0.2% cholic acid and 2% choleenamine. The cirrhosis rats were given corresponding feed for 1 week, and 55% partial cirrhosis (PH) was performed, that is, the left lobe and middle lobe of the liver of the rats were excised, and the resected tissue was washed with normal saline and weighed. On the day after operation, the rats were nursed with rewarming, sugar and saline diet.

1.2.2 Retain test specimens
The rats were sacrificed at 0, 1, 2, 3 and 7 days after operation by drawing blood from inferior vena cava under ether anesthesia. The right lobe of liver was resected, and several pieces of liver tissue with a diameter of 0.5cm were removed and immediately placed in a cryopreserved tube and stored in a refrigerator at -80°C. Attention should be paid to prevent hemolysis and coagulation during blood drawing. The blood was injected into the disposable vacuum heparin anticoagulant tube, left standing for 30 minutes, and centrifuged for 20 minutes at 2500rpm at room temperature. Suction supernatant and store at -20°C for later use. The serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), stem cell growth factor (HGF) and bile acid in each group before and after operation were determined by automatic biochemical analyzer.

1.2.3 Determination of liver regeneration:
Calculation of hepatosomatic ratio of rats after surgery: the mass of rats was recorded before preoperative anesthesia, the unit was g. The wet weight of the completely removed liver was weighed after surgery, and the wet weight of liver/liver weight *100%= hepatosomatic ratio. 1.2.4 Western blotting to detect the protein of Nil ester derivative receptor (FXR) and its target gene (CYP7A1) and FOXM1B: Total tissue protein was extracted and operated according to the reagent instructions, and the whole process was operated on ice as far as possible. 1. Protein lysate: 1MLRIPA + 1ULPSF (Benzy1 Sulfonyl Fluoride) + 1UL protein phosphatase inhibitor, mix well and put on ice for later use. 2. Cut 80mg of liver tissue into pieces, add lysate, homogenate in a tissue homogenizer, place on ice for 10 minutes, then suck the supernatant into another precooled centrifuge
tube, centrifuge at 8000g at 4 °C for 10 minutes, and then suck the supernatant into another precooled centrifuge tube to get the total protein. 3. Determination of total protein concentration: according to the instructions of the BCA kit on the total protein in the TU-1900 UV-Vis spectrophotometer, the absorbance value of 562 nm, so as to determine the concentration according to the standard curve. 4. SDS-PAGE electrophoresis, membrane transfer, sealing, primary and secondary antibodies (AbCAM, USA) incubation, exposure development (AbCAM, USA). 5. The gray value of the strips was analyzed by the gel imaging analysis system, and GAPDH was used as the internal parameter to calculate the corresponding gray value ratio of each group.

1.3 Statistical analysis
The statistical software SPSS20.0 was used to test the normal distribution of measurement data, which was represented as the mean ± standard deviation. The analysis of variance was used for the comparison of data between multiple groups, and the further pair comparison within the group was carried out by Q test. P < 0.05 was considered statistically significant.

2. RESULTS
2.1 Comparison of ALT, AST and HGF levels in three groups
At the end of surgery, ALT, AST and HGF levels of the three groups showed no statistically significant differences (P > 0.05). However, from the 3rd day after surgery, ALT and AST levels of the cholic acid-deficient group were higher than those of the cholic acid-loaded group and the control group, while HGF levels were lower than those of the cholic acid-loaded group and the control group (P < 0.05), as shown in Table 1, Table 2 & Figure 1.

Table 1. Comparison of ALT and AST levels in all the three groups

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (U/L)</th>
<th></th>
<th></th>
<th>AST (U/L)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>End of the operation</td>
<td>3rd day after surgery</td>
<td>7th day after surgery</td>
<td>End of the operation</td>
<td>3rd day after surgery</td>
<td>7th day after surgery</td>
</tr>
<tr>
<td>Cholic acid deficiency group</td>
<td>102.33±26.37</td>
<td>88.36±20.14a</td>
<td>71.26±18.74a</td>
<td>121.38±29.68</td>
<td>95.74±22.36a</td>
<td>78.36±21.47a</td>
</tr>
<tr>
<td>Cholic acid load group</td>
<td>98.69±20.14</td>
<td>69.36±15.62</td>
<td>60.29±10.32</td>
<td>124.59±27.69</td>
<td>84.36±21.69</td>
<td>68.23±14.58</td>
</tr>
<tr>
<td>Control group</td>
<td>107.85±24.69</td>
<td>60.98±12.14</td>
<td>51.39±11.47</td>
<td>120.43±26.31</td>
<td>80.66±20.46</td>
<td>60.39±13.27</td>
</tr>
<tr>
<td>F</td>
<td>1.336</td>
<td>0.011</td>
<td>0.001</td>
<td>0.691</td>
<td>0.056</td>
<td>0.001</td>
</tr>
<tr>
<td>P</td>
<td>0.215</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Note: Compared with cholic acid load group and control group, a P< 0.05

Table 2. Comparison of HGF levels in three groups

<table>
<thead>
<tr>
<th>Group</th>
<th>HGF (pg/mL)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>End of the operation</td>
<td>3rd day after surgery</td>
<td>7th day after surgery</td>
</tr>
<tr>
<td>Cholic acid deficiency group</td>
<td>142.35±21.37a</td>
<td>128.23±20.47a</td>
<td>99.65±18.62a</td>
</tr>
<tr>
<td>Cholic acid load group</td>
<td>298.62±32.58</td>
<td>267.37±26.87</td>
<td>243.12±22.01</td>
</tr>
<tr>
<td>Control group</td>
<td>237.12±27.14</td>
<td>203.99±20.14</td>
<td>187.54±17.42</td>
</tr>
<tr>
<td>F</td>
<td>20.36</td>
<td>18.96</td>
<td>23.59</td>
</tr>
<tr>
<td>P</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Note: Compared with cholic acid load group and control group, aP< 0.05
2.2 Comparison of pre-operative bile acid levels in the three groups

The total bile acid level of the rats was measured by blood before operation. The total bile acid level of the rats was (9.36±1.36) umol/L in the cholic acid deficiency group and (49.23±7.33) umol/L in the cholic acid load group and (22.39±7.21) umol/L in the control group. The difference among all groups was statistically significant (P < 0.05).

2.3 Comparison of liver regeneration in the three groups

The hepatosomatic ratio of the three groups increased with time, but the hepatosomatic ratio of the cholic acid deficiency group was lower than that of the cholic acid load group and the control group (P < 0.05), as shown in Table 3.

Table 3. Comparison of hepatosomatic ratio of three groups of rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>End of the operation</th>
<th>3rd day after surgery</th>
<th>7th day after surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholic acid deficiency group</td>
<td>1.23±0.04</td>
<td>1.89±0.07</td>
<td>2.31±0.15</td>
</tr>
<tr>
<td>Cholic acid load group</td>
<td>1.16±0.08</td>
<td>2.25±0.26</td>
<td>3.15±0.34</td>
</tr>
<tr>
<td>Control group</td>
<td>1.20±0.07</td>
<td>2.13±0.36</td>
<td>3.08±0.26</td>
</tr>
<tr>
<td>F</td>
<td>0.69</td>
<td>6.325</td>
<td>5.871</td>
</tr>
<tr>
<td>P</td>
<td>0.062</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

2.4 Comparison of expression levels of FXR target genes CYP7A1 and FOXM1B in three groups of rats

On the 7th day after surgery, the expression of the target genes of FXR, CYP7A1 and FOXM1B increased, and the content of FOXM1B in the cholic acid deficiency group was the highest, with FOXM1B up to 2.33±0.36, which showed statistical significance compared with the control group and the cholic acid load group (P < 0.05), while the expression of FXR was the lowest in the cholic acid deficiency group (P < 0.05), as shown in figure 3.
FXR is the earliest identified bile acid receptor, widely expressed in liver, small intestine, fat, heart and other organs. It maintains the metabolic balance of bile acids by regulating the expression of genes involved in the metabolism of bile acids. It has certain inhibitory effects on the synthesis of bile acids, accelerating the detoxification and excretion of bile acids, and regulating the transport of bile acids. Thus, liver tissue damage caused by excessive bile acid load can be reduced. 6-7 Rania 8 found that the participation of bile acid in liver regeneration mainly depended on the completion of FXR, but after partial liver resection, 0.2% cholic acid feed had a significant effect on liver regeneration; on the contrary, the speed of liver regeneration could be reduced by reducing the level of bile acid. Studies at home and abroad have reported that bile acids are an important cell promoter and a general term for a class of bile alkanic acids. Cholesterol can be metabolized into bile acids in the body, and FXR, the main receptor of bile acids, can promote liver regeneration when residual liver cells are exposed to increased load of bile acids. 9-11 Therefore, it is believed that FXR plays a key role in liver regeneration by regulating bile acid signaling pathway. By transforming bile acid stress signal into a driving force to promote liver regeneration, FXR plays a role in inhibiting bile acid synthesis and promoting DNA repair in damaged liver cells. However, the molecular mechanism of how FXR mediates bile acid to promote liver regeneration has not been clearly reported at present.12

CYP7A1 is a major regulatory gene of bile acid metabolism. The synthesis of liver cholic acid mainly includes two pathways, namely classical synthesis pathway and bypass synthesis pathway. The rate-limiting enzyme in the classical synthesis pathway is CYP7A1, which mainly synthesizes (binding cholic acid) CA and (glycodeoxycholic acid) CDCA, the primary bile acid. Studies have shown that FXR can inhibit the synthesis of the rate-limiting enzymes CYP7A1 and CYP8B1, thereby reducing the damage of bile acids to the liver and promoting liver regeneration. In addition, FXR can also affect liver regeneration by regulating the expression of transcription factor FOXM1B. FOXM1B, as a specific transcription factor, is significantly elevated in cell proliferation. FOXM1B is an important target gene of FXR involved in cell cycle regulation. FXR mediates cell cycle mitosis by inhibiting FOXM1B expression.13-14 Therefore; our study will focus on the molecular biological mechanism of FXR mediated bile acid pathway to promote liver regeneration, to provide a reference for clinical treatment.

In our study, the expression of FXR and CYP7A1, liver regeneration, ALT, AST, ALB synthesis and the influence of endotoxin were observed by changing the presence of cholic acid in cirrhosis rats, so as to determine the role of endotoxin and nuclear receptor-dependent bile acid signals in regulating residual liver regeneration after partial hepatectomy of sclerosing liver.

We found that the hepatosomal ratio of the three groups increased with time, but the hepatosomal ratio of the cholic acid deficiency group was lower than that of the cholic acid load group and the control group. Hepatosomal ratio is an objective and effective indicator of liver regeneration, which not only takes into account the change of body weight during liver regeneration, but also reflects the macro expression of the increase of residual liver volume. This study suggested that liver regeneration was more obvious in the bile acid load group with the extension of time. Further comparison of ALT, AST and HGF levels in each group showed that from the 3rd day after surgery, ALT and AST levels in the cholic acid deficient group were higher than those in the cholic acid-loaded group and the control group, while HGF levels were lower than those in the cholic acid-loaded group and the control group.
Studies suggest that when most of the resection of liver, the rest of the normal liver tissue blood supply were increased and prone to the liver tissue perfusion in damage, degeneration necrosis of hepatic cells, and ALT, AST is a sensitive indicator of liver function change \(^{12}\), this study suggests that bile acid load group of liver cell regeneration is more apparent, with extended time, reduce liver damage, Liver function recovery is accelerated, ALT and AST will gradually return to normal. As a strong stimulant of cell mitogen, HGF is a sensitive indicator of liver cell regeneration, which is consistent with the conclusion of this study. In our study, the changes of target genes of FXR were further examined. The results showed that the expressions of the target genes of FXR, CYP7A1 and FOXM1B, were increased on the 7th day after surgery, and the contents of the target genes of FXR were the highest in the cholic acid deficiency group, while the expression of FXR was the lowest in the cholic acid deficiency group. The results indicated that FXR played a key regulatory role in mediating bile acid pathway to promote liver regeneration. Bile acids play a significant role in regulating liver regeneration through activation of related signaling pathways. Our study preliminarily confirmed that CYP7A1 and FOXM1B are important target genes in FXR mediated bile acid metabolism and regulation. This study will provide important ideas for liver surgery and liver transplantation.

**CONCLUSION**

Our study concluded that maintaining normal intestinal bile acid content plays a key role in FXR mediated bile acid signaling to promote liver regeneration, and also provides a certain basis for the clinical application of cholic acid in perioperative hepatectomy of sclerosing liver.

**Conflict of Interest:** Nil

**Funding:** Nil

**REFERENCE**