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Full Length Research Paper

Effect of Fufang Jiangzhi No. 3 on cholesterol-bile acid metabolism in New Zealand white rabbit fed with cholesterol-rich diet

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The effect of Fufang Jiangzhi No. 3 on cholesterol-bile acid metabolism in New Zealand white rabbit fed with cholesterol-rich diet was studied. 24 male New Zealand white rabbits were randomly assigned into control group (Group A), hypercholesterolemia model group (Group B), and Fufang Jiangzhi No. 3 treatment group (Group C). Groups B and C were fed with cholesterol-rich diet (containing 1% cholesterol) 120 g/day during 4 weeks' administration in order to establish hypercholesterolemia model while Group A was fed with common rabbit fodder 120 g/day. Group C received Fufang Jiangzhi No. 3 by intragastric administration (0.5 bag/20 ml distilled water, every morning) at the same time as the start of the cholesterol-rich diet exposure. Serum CHO, LDL-C and BA assessment of 24 rabbits was performed at the end of the experiment. The activity of CYP7A1 in the liver was measured by enzyme-linked immunosorbent assay (ELISAs). The expressions of CYP7A1 mRNA, bile salt export pump (BSEP) mRNA and small heterodimer partner (SHP) mRNA in the liver were measured by real time polymerase chain reaction (RT-PCR). Serum CHO in Group B was much higher than that in Group A ($P<0.05$), moreover, the serum CHO in Group C was lower than that in Group B ($P<0.05$). The level of BSEP mRNA and SHP mRNA in Group C were much lower than those of Group B ($P<0.01$). These results suggested that Fufang Jiangzhi No. 3 can up-regulate the expression of CYP7A1 mRNA and enhance the activity of CYP7A1. It may be one of the mechanisms involved in its preventive effect in cholesterol-rich diet-induced hyperchlesterolemia in New Zealand white rabbit.

Key words: Bile acid, Fufang Jingzhi No. 3, Farnesoid X Recptor (FXR), CYP7A1.

INTRODUCTION

The liver is the main organ for cholesterol metabolism, where cholesterol is changed into bile acid as the final metabolite (Chiang, 2002). The nuclear receptor (Farnesoid X Recptor, FXR) plays an important role in the cholesterol-bile acid metabolism. Bile salt export pump (BSEP) and small heterodimer partner (SHP) are the important up-regulation target genes for FXR. FXR regulates the expression of SHP mRNA, and SHP is a strong transcription inhibitory factor of CYP7A1, which is the key enzyme for the metabolism of cholesterol and bile acid; meanwhile, the level of BSEP mRNA can reflect the level of FXR mRNA in the organism. Fufang Jiangzhi No. 3, which consists of *Astragalus mongholicus*, *Rhizoma alismatis*, *Rhizoma coptidis*, *Radix curcumae*, *Cattail pollen*, *Hawthorn* and *Maltum*, is developed by the Department of Traditional Chinese Medicine, Huashan Hospital, Fudan University to reduce the cholesterol level of hyperlipoidemia patient.

In our present work, the 24 rabbits were fed with cholesterol-rich diet in order to establish hyper-cholesterolemia model. The activity of CYP7A1 in the liver was measured by enzyme-linked immunosorbent assay (ELISAs). The expressions of CYP7A1 mRNA, BSEP mRNA and SHP mRNA in the liver were measured

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by RT-PCR. In a word, this study was conducted to investigate the possible mechanism of the compound decreasing the level of blood cholesterol by regulating cholesterol-bile acid metabolism.

MATERIALS AND METHODS

Modeling and animal grouping

24 male New Zealand white rabbits (body weight 2.5 to 3.0 kg) used for this experiment were purchased from Fudan University, housed individually in stainless steel mesh-bottomed cages at room temperature (RT), fed with common rabbit fodder; each rabbit was fed with about 120 g/day. The rabbits were randomly assigned into control group (group A), hypercholesterolemia model group (Group B) and Fufang Jiangzhi No. 3 treatment group (Group C), after being raised one week in Experimental Animal Center of Fudan University. The rabbits in group A were fed with common rabbit fodder, while in Groups B and C, they were fed with the basal diet plus 1% of pure cholesterol. At the same time, the rabbits in groups A and B were intragastricly administrated with 20 ml distilled water every morning. Group C received Fufang Jiangzhi No. 3 by intragastric administration (0.5 bag dissolved in 20 ml distilled water, every morning) during the same period.

Specimen collection

At the end of the experiment, 2 ml of blood were collected from each rabbit. After blood collection, serums were allowed to repose for 30 min at RT and thus slightly centrifuged in a bench centrifuge for 10 min at RT. The clean samples were finally stored at -20°C until use. The serum was used to determine the CYP7A1 activity and mRNA, SHP mRNA and BSEP mRNA of rabbits.

Determination of serum cholesterol level

Serum cholesterol level was determined by enzyme hydrolysis. The cholesterol assay kit was purchased from Shanghai Mindian Biological Engineering Co., Ltd., and conducted with the Olympus AU600 automatic biochemical analyzer.

Determination of serum bile acid level

Serum bile acid level was determined by circulating enzymatic method, automatic bile acid assay kit purchased from Japan Chemicals Co., Ltd., and conducted with the Olympus AU600 automatic biochemical analyzer.

Determination of hepatic CYP7A1 activity

Hepatic CYP7A1 activity was measured by ELISA, rabbit cholesterol 7α2 hydroxylase activity assay kit purchased from American ADL company, and conducted with the Bio-Tek ELX800 Universal Microplate Reader.

Real time polymerase chain reaction (RT-PCR)

Total RNA was isolated by one-step method using the Trizol reagent (ABI). After RNA reverse transcription, SYBRGreen PCR kit was applied in PCR amplification, using ABI PRISMR7300 instrument (ABI) for testing. Table 1 shows the primers used for each gene amplification, PCR reaction volume was 50 µl, with GAPDH as internal reference. Data was supplied by ABI Prism 7300 SDS analysis software.

Statistical analysis

All results were expressed as mean ± SEM. Data were analyzed by one-way ANOVA using SPSS. *p* values less than 0.05 were considered statistically significant.

RESULTS

Bile acid and cholesterol levels in peripheral blood

At the end of the experiment, serum CHO levels in Groups A, B and C were 4.51±0.45, 23.14±3.21 and 16.21±2.14 mmol/L, respectively. Serum CHO in Groups B and C were much higher than that in Group A ($P<0.01$); moreover, the serum CHO in group C was lower than that in Group B ($P<0.01$). Serum bile acid levels in Groups A, B and C were 7.45±1.33, 13.95±1.86 and 13.31±1.27 µmol/L, respectively (Table 2). Serum bile acid in groups B and C were much higher than that in group A ($P<0.01$). There was no significant difference of serum bile acid levels between groups B and C ($P> 0.05$).

Hepatic CYP7A1 activity and CYP7A1 mRNA levels

At the end of the experiment, the concentrations of CYP7A1 in hepatic samples of the three groups were 0.80±0.22, 0.44±0.16 and 0.81±0.21 µg/ml, respectively. The concentration of Group B was much lower than that of Group A ($P<0.01$). The difference of the concentrations of CYP7A1 in hepatic samples of Groups A and C has no statistical significance ($P> 0.05$). The concentration of Group C was much higher than that of group B ($P<0.01$). 4 weeks later, the expressions of CYP7A1 mRNA in hepatic samples of three groups were 1.97±1.50, 0.70±0.27 and 3.87±1.68, respectively (Table 3). The CYP7A1 mRNA level of Group B was much lower than that of Group A ($P<0.01$) .The CYP7A1 mRNA level of Group C was higher than that of groups B and A ($P<0.01$).

Liver BSEP mRNA and SHP mRNA levels

At the end of the experiment, the expressions of BSEP mRNA in hepatic samples of the three groups were 7.33±2.44, 16.40±3.01 and 9.64±1.63, respectively (Table 4). The expressions of SHP mRNA in hepatic samples of the three groups were 9.83±2.50, 85.44±6.79 and 20.73±4.47, respectively. The levels of BSEP mRNA and SHP mRNA in group B were much higher than those of Group A ($P<0.01$). The levels of group C were lower than those of Group B ($P<0.01$). The levels of Group C were

Table 1. Primers used for each gene amplification.

Gene	Primer sequences	Temperature (℃)	Length
SHP	F 5'-TGGCCCAAGACATGGTGAC-3' R 5'-GCTCCTCCAGCAGGATCTTCT-3'	57	115
BSEP	F 5'- CAACGCATTGCTATTGCTCG-3' R 5'-GTTCTGGATGGTGGACAAACG-3'	57	130
CYP7A1	F 5'-AGGAGAAGGCGAATGGGTGC-3'	57	151
GAPDH	F 5'-CCGAGGGCCCACTAAAGG－3' R 5'-GCTGTTGAAGTCACAGGAGA－3'	57	166

Table 2. The level of serum CHO and serum BA for each group.

Group	n	Serum CHO (mmol/L)	Serum BA (μmol/L)
A	8	4.51 ± 0.45	7.45 ±1.33
B	8	23.14 ± 3.21*	13.95 ± 1.86*
C	8	16.21 ± 2.14*#	13.31± 1.27*+

Table 3. Hepatic CYP7A1 activity and CYP7A1 mRNA levels for each group.

Group	n	CYP7A1 activity (μg/ml)	CYP7A1 mRNA
A	8	0.80 ± 0.22	1.97 ± 1.50
B	8	0.44 ± 0.16*	0.70 ± 0.27*
C	8	0.81 ± 0.21#	3.87 ± 1.68*#

*p<0.01, compared with group A; #p<0.01, compared with group B.

Table 4. The expressions of SHP mRNA and BSEP mRNA in hepatic tissue.

Group	n	SHP mRNA	BSEP mRNA
A	8	9.83 ± 2.50	7.33 ± 2.44
B	8	85.44 ± 6.79*	16.40 ± 3.01*
C	8	20.73 ± 4.47*#	9.64 ± 1.63*#

*p<0.01, compared with group A; #p<0.01, compared with Group B.

higher than those of Group A (P<0.01).

DISCUSSION

This study shows that bile acid is a natural excitability FXR ligand (Abrahamsson et al., 2005). When FXR and bile acid combined, its space structure changed, then it combined with RXR to form heterodimers (FXR/RXR), which was to be combined with the SHP gene regulatory region to increase the expression of SHP mRNA. SHP is a transcription inhibitor, which is combined with CYP7A1 gene regulatory region to form a repressive complex, and reduce the expression of CYP7A1 mRNA, then reduce the CYP7A1 activity. CYP7A1 is the key enzyme of cholesterol-bile acid metabolism, so the lowering activity will cause that the transformation from cholesterol to bile acid be reduced in the liver (Xu et al., 2004). FXR, which acts as the upstream regulatory factor, has the important conditioning in the cholesterol-bile acid metabolism. It is not direct, and plays a role indirectly by way of SHP. BSEP and SHP are both the up-regulation target genes for FXR. The level of BSEP mRNA and SHP mRNA is to reflect the change of the level of FXR mRNA. Bile acid metabolized from cholesterol is the important path of cholesterol metabolism, so FXR can play the

accommodation of cholesterol with controlling the cholesterol-bile acid metabolism.

In the present study, the New Zealand white rabbits were fed with the cholesterol-rich diet which contained 1% cholesterol for 4 weeks, and the rabbits never had diarrhea, loss of appetite or died. At the end of the experiment, the cholesterol level in Group B was obviously higher than that in Group A (P<0.01), and the cholesterol level in Group C was much lower than that in Group B and higher than that in Group A (P<0.01). It shows that the hypercholesterolemia models were induced by feeding with cholesterol-rich diet for 4 weeks, and the rabbits had light adverse effects while no death occurred. The intragastric administration of Fufang Jiangzhi No. 3 to the rabbits had the intervention role for the forming of hypercholesterolemia

Our results showed that the expression level of BSEP and SHP mRNA of Group B were obviously higher than those of Group A (P<0.01), and the expression level of CYP7A1 mRNA and activity of CYP7A1 of Group B were much lower than those of Group A (P<0.01). Meanwhile, the expression levels of BSEP and SHP mRNA of Group C were obviously higher than those of Group B (P<0.01), and the expression level of CYP7A1 mRNA and activity of CYP7A1 of Group C were much lower than those of Group B (P<0.01). These results indicated that the FXR were activated obviously when rabbits were fed with cholesterol-rich diet, which was in term of the increased expression of BSEP and SHP mRNA. Since high cholesterol diet, the synthesization of bile acid increased in the liver. Then, the bile acid activated FXR as natural FXR excitability ligand. The activated FXR suppressed the expression of CYP7A1 mRNA by SHP to decrease the activity of CYP7A1. As stated previously, FXR's activating was likely to play an important part in hypercholesterolemia's forming process caused by cholesterol-rich diet (Suzuki et al., 2008; Wang et al., 2008). When fed by intragastric administration with Fufang Jiangzhi No. 3, the rabbits' FXR activity were suppressed, which can be proved by the expression level of BSEP mRNA and SHP mRNA of Group C which were obviously higher than those of Group B. The interfering effect and mechanism of Fufang Jiangzhi No. 3 to hypercholesterolemia may be the decreasing of the transcription inhibition from FXR to CYP7A1 by suppressing FXR's activation, and increasing the expression of CYP7A1 mRNA to enhance the activity of CYP7A1, then promoting cholesterol synthesizing bile acid in the liver, which causes the liver to uptake cholesterol from periphery, then decreasing the peripheral cholesterol levels.

Since the synthesization of bile acid from cholesterol was enhanced by Fufang Jiangzhi No. 3, however, there was no significant difference in the level of bile acid between groups B and C. We proposed that Fufang Jiangzhi No.3 may activate the excretory mechanism of bile acid while it promotes the synthesizing. BSEP plays an important role in the process of bile acid entering into bile capillaries by liver cells and then entering the gallbladder. The activation of FXR increased the expression level of BSEP mRNA, and promoted the bile acid entering into the biliary system and then being excreted out of the body from the alimentary canal, thus preventing toxic damage by accumulation of bile acid. Of course, there may be other mechanisms of promoting the excretion of bile acid to maintain the stabilization of total bile acids.

Conclusion

In conclusion, the activation of FXR by cholesterol-rich diet may be the mechanism of the rabbits hypercholesterolemia. Fufang Jiangzhi No. 3 obviously inhibited the synthesization of hypercholesterolemia in the rabbits. The mechanism might be related to inhibited activation of FXR to decrease the expression level of SHP mRNA and the activity of SHP, then decreasing the transcription inhibition of CYP7A1 to increase the expression of CYP7A1 mRNA and enhance the activity of CYP7A1, then, promoting cholesterol synthesizing bile acid in the liver, which induced a decrease of the peripheral cholesterol levels.

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