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Effects of *Clostridium butyricum* Capsules Combined with Rosuvastatin on Intestinal Flora, Lipid Metabolism, Liver Function and Inflammation in NAFLD Patients

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ABSTRACT

The objective of this study was to investigate the effects of *Clostridium butyricum* capsules combined with rosuvastatin on the intestinal flora, lipid metabolism, liver function and inflammation in patients with nonalcoholic fatty liver disease (NAFLD). For this purpose, a total of 96 patients with NAFLD were selected as research subjects and randomly divided into a control group (n=48) and an observation group (n=48). The Control group was treated with rosuvastatin, based on which observation group received *Clostridium butyricum* capsule treatment. The efficacy in the two groups of patients was compared, and the intestinal flora, lipid metabolism, liver function and inflammation were observed. Results showed that the efficacy in the observation group was significantly better than that in the control group (p<0.05). After treatment, the content of Eubacterium rectale in the observation group was lower than that in the control group, while the content of Bacteroides thetaiotaomicron and Bifidobacteria was notably higher than that in the control group (p<0.05). Moreover, the observation group had remarkably lower levels of total cholesterol (TC), triglyceride (TG), free fatty acids (FFA), total bilirubin (TBIL), direct bilirubin (DBIL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), procollagen III peptide (PIIIP), collagen-IV (C-IV), hyaluronicacid (HA) and laminin (LN) as well as lower levels of tumor necrosis factor-alpha (TNF-α), catabolite activator protein (CAP) and interleukin-6 (IL-6) in serum than the control group (p<0.05). It was concluded that Clostridium butyricum capsules combined with rosuvastatin can effectively improve intestinal flora imbalance, reduce blood lipid levels, and alleviate liver fibrosis and liver function damage in the treatment of NAFLD, so it is of therapeutic value.

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Introduction

Nonalcoholic fatty liver disease (NAFLD), a clinical common liver disease, has a relatively high incidence rate (1). As modern society constantly progresses, and people's living standards have been improved, the incidence rate of NAFLD shows a year-by-year increasing trend, and NAFLD patients tend to be younger (2). Such factors as intestinal flora, lipid metabolism and inflammatory reaction all participate in the pathogenetic process of NAFLD. Besides, failure to treat NAFLD in time can accelerate liver fibrosis, aggravate liver injury and induce liver cirrhosis. When NAFLA develops into the decompensation stage, it is prone to canceration and progression into liver cancer, thus seriously threatening human health (3-4). Therefore, improving intestinal flora and lipid metabolism of patients through timely and effective treatment of NAFLD can better promote the rehabilitation of NAFLD patients. In this study, clostridium butyricum capsules combined with rosuvastatin were given to NAFLD patients to observe the changes in the intestinal flora, lipid metabolism, liver function and inflammation, thereby providing a theoretical basis for the prevention and treatment of NAFLD.

Materials and methods

General data

A total of 96 NAFLD patients, were admitted to the department of General medical, Tongren Hospital, Shanghai Jiaotong University of Medicine and Department of Gastroenterology, Zhongshan Medical College, Qingpu, Fudan University from January 2019 to January 2020, were randomly divided into control

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group and observation group. The division was based on the following inclusion criteria: 1) patients who met NAFLD diagnostic criteria [5], 2) patients who stopped taking NAFLD drugs one month before inclusion, and (3) patients who signed the informed consent form. The division was based on the following exclusion criteria: 1) patients with severe heart, liver and renal dysfunction, 2) patients with autoimmune hepatitis, liver cancer or viral hepatitis, or 3) patients who were allergic to the drugs applied in this study. Then these patients were randomly allocated into the control group (n=48) and observation group (n=48). No significant differences in general data were detected between the two groups (p>0.05) (Table 1).

Table 1. General data of the two groups of patients

Item	Observation group (n=48)	Control group (n=48)	t/χ^2	p
Age (years old)	32-67	33-66		
Gender (male/female)	28/20	26/22	0.042	0.837
Average age (years old)	47.73±8.42	48.18±8.73	0.257	0.798
BMI (Kg/m^2)	23.75±1.56	23.56±1.53	0.602	0.549
Degree of education				
Junior high school and below	11 (22.92)	10 (20.83)		
High school and technical secondary school	21 (43.75)	23 (47.92)	0.171	0.918
Junior college or above	16 (33.33)	15 (31.25)		
Smoking [n (%)]	13 (27.08)	15 (31.25)	0.051	0.822
Drinking [n (%)]	18 (37.50)	20 (41.64)	0.044	0.835

Treatment methods

The Control group received oral medication of rosuvastatin [manufacturer: Zhejiang Jingxin Pharmaceutical Co., Ltd, Approval No.: NMPN H200804838] twice a day, 10 mg/time. The observation group was treated with clostridium butyricum capsules [manufacturer: Chongqing Taiping Pharmaceutical Co., Ltd., Approval No.: NMPNS20040054] for 3 times a day, 400 mg/time, based on the treatment in the control group. Both groups of patients were treated for 6 months.

Detection of intestinal flora

Feces samples (2-3 g) were collected from the two groups of patients, sub-packaged into Eppendorf tube (EP) tubes and stored at -80°C. Then 0.5 g of feces samples were mixed with 10 mL of PBS solution, shaken well and centrifuged (3000 r/min) for 5 min to take supernatant. The centrifugation was further repeated twice to take supernatant, which was labeled as Supernatant 1. Next, 1 mL of Supernatant 1 was put into EP tubes and centrifuged (12000 r/min) for 10 min. Later, the supernatant was discarded, and another 1 mL of supernatant was added and centrifuged (12000 r/min) for 10 min twice. Finally, precipitates were taken. In strict accordance with the instructions of the bacterial genomic deoxyribonucleic acid (DNA) extraction kit (Hangzhou Guhe Information Co., Ltd.), the bacterial total DNA was extracted from precipitates. After that, a routine polymerase chain reaction (PCR) was carried out with the genomic DNA extracted from feces of normal people as a template, and a highly efficient centrifugal column agarose gel DNA recovery kit （Hangzhou Guhe Information Co., Ltd) was adopted for purification and recovery of DNA, which was regarded as a standard substance to plot standard curves of Eubacterium rectale, Bacteroides thetaiotaomicron and Bifidobacterium. Subsequently, bacterial DNA samples in feces to be tested in the two groups of patients were simultaneously subjected to real-time fluorescence quantitative PCR, and amplification curves were drawn. After the reaction, the copy number of bacterial genes to be tested was automatically analyzed by the software of the PCR instrument as a quantitative result.

Detection of related indexes

Fasting venous blood was collected in the morning before and after treatment, centrifuged (3000 r/min) for 15 min to collect supernatant and stored in a refrigerator at -80°C for later detection. After that, the levels of total bilirubin (TBIL), direct bilirubin (DBIL), aspartate aminotransferase (AST) and aspartate aminotransferase (ALT) in serum were detected using a full-automatic biochemical analyzer (Siemens advia240). Then enzyme-linked immunosorbent assay (ELISA) was applied to examine the levels of hyaluronic acid (HA), procollagen III peptide (PIIIP), collagen-IV (C-IV), the liver fibrosis index laminin (LN), tumor necrosis factor-alpha (TNF-α), catabolite activator protein (CRP) and interleukin-6 (IL-6) in serum of patients using relevant kits provided by Qingdao Driskell Biotechnology Co. LTD. in strict accordance with the instructions.

Evaluation of curative effects

Criteria for efficacy (6): 1) Markedly effective: After treatment, the symptoms of the patient disappear, and blood lipid and liver function are improved by more than 50%, and B-ultrasound examination shows no fatty liver. 2) Effective: After treatment, the symptoms of the patient are relieved, and the blood lipid and liver function are improved by 10-50%, and B-ultrasound examination manifests slight enhancement of liver echo scattering. 3) Ineffective: After treatment, the patient's symptoms are not significantly alleviated or worsened, and blood lipid and liver function are improved by <10%, and no evident changes are shown in B-ultrasound examination.

1.4 Statistical processing

SPSS 19.0 software (SPSS Inc., Chicago, IL, USA) was utilized for data processing. Measurement data were expressed as mean ± standard deviation ($\bar{x}\pm s$) and detected via *t*-test. Count data were reflected as a percentage and examined using the χ^2 test. $p<0.05$ represented that the difference was statistically significant.

Results and discussion

Comparison of clinical efficacy between the two groups of patients

The effective rate of treatment in the observation group was higher than that in the control group ($p<0.05$) (Table 2).

Table 2. Comparison of clinical efficacy between the two groups of patients [n (%)]

Group	n	Markedly effective	Effective	Ineffective	Effective rate
Control group	48	10 (20.83)	18 (37.50)	20 (41.67)	28 (58.33)
Observation group	48	22 (45.83)	20 (41.67)	6 (12.50)	42 (87.50)
χ^2					8.914
p					0.003

Comparison of intestinal flora between the two groups of patients

Following treatment, the control group had a remarkably higher content of Eubacterium rectale and notably lower content of Bacteroides thetaiotaomicron and Bifidobacterium than the observation group ($p<0.05$) (Table 3).

Table 3. Comparison of intestinal flora between the two groups of patients after treatment

Group	n	Eubacterium rectale ($\times10^3$)	Bacteroides thetaiotaomicron ($\times10^3$)	Bifidobacterium ($\times10^6$)
Control group	48	3.83±1.58	2.93±1.57	2.68±1.46
Observation group	48	2.35±1.49	4.15±1.46	9.85±2.15
t		4.721	3.942	19.114
p		<0.001	0.001	<0.001

Comparison of blood lipid metabolism between the two groups of patients

The levels of total cholesterol (TC), triglyceride (TG) and free fatty acids (FFA) after treatment in the observation group were prominently lower than those in the control group ($p<0.05$) (Table 4).

Table 4. Blood lipid metabolism in the two groups of patients

Group	n	TG (mmol/L)	TC (mmol/L)	FFA (mmol/L)
Control group	48	4.08±1.03	5.21±1.18	0.87±0.37
Observation group	48	3.02±0.89	4.05±1.03	0.35±0.12
t		5.395	5.131	9.262
p		<0.001	<0.001	<0.001

Comparison of liver function indexes between the two groups of patients

As shown in Table 5, the observation group showed lower levels of serum TBIL, DBIL, ALT and AST than the control group following treatment ($p<0.05$).

Table 5. Comparison of liver function indexes between the two groups of patients after treatment

Group	n	TBIL (μmol/L)	DBIL (μmol/L)	ALT (U/L)	AST (U/L)
Control group	48	37.24±5.69	18.91±5.23	36.88±7.93	42.55±9.22
Observation group	48	30.20±5.38	11.24±4.53	26.37±6.55	31.58±9.64
t		6.229	7.680	7.080	5.698
p		<0.001	<0.001	<0.001	<0.001

Comparison of serum liver fibrosis indexes between the two groups of patients

It was displayed in Table 6 that the levels of serum PIIIP, C-IV, HA and LN in the observation group were lower than those in the control group ($p<0.05$).

Table 6. Comparison of serum liver fibrosis indexes between the two groups of patients after treatment

Group	n	PIIIP (μg/L)	C-IV (μg/L)	HA (μg/L)	LN (μg/L)
Control group	48	183.46±13.27	98.14±9.38	232.46±23.79	153.53±12.65
Observation group	48	108.83±13.14	74.45±7.07	105.53±12.37	87.42±9.38
t		27.687	13.973	32.796	28.644
p		<0.001	<0.001	<0.001	<0.001

Comparison of serum inflammatory factors between the two groups of patients

Table 7 reveals that the levels of serum TNF-α, CRP and IL-6 in the observation group are lower than those in the control group (p<0.05).

Table 7. Levels of serum inflammatory factors in the two groups of patients following treatment

Group	n	TNF-α (pg/mL)	CRP (mg/L)	IL-6 (ng/L)
Control group	48	25.63±3.56	3.26±0.68	12.53±2.38
Observation group	48	17.37±3.54	1.24±1.36	6.82±1.75
t		11.399	9.204	13.391
p		<0.001	<0.001	<0.001

NAFLD is a common clinical liver disease and a metabolic syndrome resulting from non-alcohol factors, which is mainly manifested as diffuse macrovesicular steatosis of liver cells (7-8). Without timely diagnosis or treatment, NAFLD may develop into liver fibrosis, thus leading to liver cirrhosis and even canceration, which seriously threatens the life and health of patients (9). At present, the pathogenesis of NAFLD is not entirely understood due to its correlations with lipid peroxidation damage, insulin resistance, oxidative stress, intestinal flora imbalance, inflammatory responses and other factors (10,11). Therefore, it is indispensable to regulate the imbalance of intestinal flora in the treatment process of NAFLD.

NumerousHuman intestinal flora can be classified into beneficial bacteria, harmful bacteria and neutral bacteria. Such flora is combined and balanced in a certain proportion in the human intestinal tract to form the intact intestinal mucosa, to prevent the invasion of toxic substances in the intestinal cavity and act as a biological barrier and immune barrier (12). Eubacterium rectale, a member of Firmicutes, is a conditional pathogen that is able to ferment glucose metabolites (formic acid, acetic acid and butyric acid) and proteins, to reduce glycan-degrading enzymes and suppress the proliferation of other beneficial bacteria (13). Bacteroides thetaiotaomicron is a Gram-negative bacterium belonging to Bacteroides, and it can synthesize vitamins and proteins, assist the normal absorption of food, and facilitate the maintenance of the intestinal microecological balance (14). Bifidobacterium is a Gram-positive bacterium that is beneficial to human health (15). The results of this study showed that the observation group had lower content of Eubacterium rectale and higher content of Bacteroides thetaiotaomicron and Bifidobacterium than the control group after treatment (p<0.05). This may be due to the fact that clostridium butyricum capsules can be directly colonized in the intestinal tract and start mass reproduction after oral administration. In addition, they have an inhibitory effect on the reproduction of harmful bacteria such as Eubacterium rectale. They co-exist with probiotics, such as Bacteroides thetaiotaomicron and Bifidobacterium, to promote their proliferation, thus optimizing the intestinal flora structure and improving the intestinal flora disorder in NAFLD patients.

The lipid metabolism in NAFLD patients is relatively disorganized, which increases the burden on the liver, causes a "second strike" on the liver, and aggravates the damage to the liver and the disease in patients (16). FFA is the material basis of NAFLD formation, which can reflect the liver's ability to transport triglycerides and the body's insulin resistance (17). PIIIP, C-Ⅳ, HA and LN are liver fibrosis indexes commonly used in the clinic (18). HA, a polymer polysaccharide is the main component of the basement membrane. It can be synthesized and secreted by hepatic stellate cells in large quantities when liver fibrosis occurs, thus promoting the capillarization of hepatic sinusoids (19). The results of this study revealed that the levels of serum TC, TG, FFA, TBIL, DBIL, ALT, AST, PIIIP, C-IV, HA and LN in the observation group were significantly lower than those in the control group (p<0.05). The reason may be that the combination of clostridium butyricum capsules and rosuvastatin can enhance the liver metabolic ability of NAFLD patients, which further accelerates lipid metabolism, improves the triglyceride transportation and ability to treat FFA, and reduces the degree of insulin resistance in the body.

Inflammation has been proved to be closely associated with the occurrence and development of NAFLD. Disturbance of intestinal flora in NAFLD patients will lead to an increase in intestinal endotoxin, which will cause intestinal endotoxemiaeasily. Besides, it can stimulate the releases of TNF-α, CRP, IL-6 and other inflammatory factors in large quantities and amplify the inflammatory reaction, thus causing a second strike on liver cells and inflammatory damage to the liver (20-

22). The results of this study demonstrated that after treatment, the levels of serum TNF-α, CRP and IL-6 in the observation group were lower than those in the control group (p<0.05). The results may be explained by the positive regulatory effect of Clostridium butyricum capsules on the intestinal microecology balance of patients. These capsules modulate T cell and B cell subsets to reduce the releases of TNF-α, CRP and IL-6 and alleviate the intestinal inflammatory reaction, thereby indirectly protecting the liver of NAFLD patients.

To sum up, combined therapy of *C. butyricum* capsules and rosuvastatin for NAFLD patients can ameliorate the intestinal flora imbalance, regulate lipid metabolism and decrease the production of inflammatory factors, thus improving the liver function of patients and providing new ideas for the prevention and treatment of NAFLD.

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Conflict interest

The authors declare no conflict of interest.

References

1. Alessandro Mantovani. Nonalcoholic Fatty Liver Disease (NAFLD) and Risk of Cardiac Arrhythmias: A New Aspect of the Liver-heart Axis. J Clin Transl Hepatol 2017; 5(2):134-141.
2. Tateishi R, Koike K. Epidemiology and risk factors of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH). Nihon Shokakibyo Gakkai Zasshi 2017;114(5):813-8.
3. RehamAbdou, Susan S. Baker, Robert D. Baker. Hepatic Flavin Monooxygenase 3 (FMO3) is Highly Up Regulated in Nonalcoholic Fatty Liver Disease (NAFLD). Gastroenterol 2017; 152(5):S1156-S1157.
4. De Assunção SN, Sorte NC, Alves CD, Mendes PS, Alves CR, Silva LR. Nonalcoholic fatty liver disease (NAFLD) pathophysiology in obese children and adolescents: update. Nutrición Hospitalaria. 2017;34(3):727-30.
5. Bernard Fromenty. Role of nonalcoholic fatty liver disease as risk factor for drug-induced hepatotoxicity. J ClinTransl Res 2017; 3(1):212-232.
6. Jiang Y, Han T, Zhang ZG. Potential role of thymosin beta 4 in the treatment of nonalcoholic fatty liver disease. Chronic Dis Transl Med 2017; 3(3):165.
7. Lan N. Tu, Megan R. Showalter, Tomas Cajka. Metabolomic characteristics of cholesterol-induced non-obese nonalcoholic fatty liver disease in mice. Sci Rep 2017; 7(1):6120.
8. George S. Riad, Hany H. Kaisar, Hany A. Hussein. The relation between nonalcoholic fatty liver disease and carotid intima-media thickness in Egyptian patients. Egypt Liver J 2017; 7(1 and 2):21-25.
9. Ui-Jin Bae, Eun-Ock Park, John Park. Gypenoside UL4-Rich Gynostemmapentaphyllum Extract Exerts a Hepatoprotective Effect on Diet-Induced Nonalcoholic Fatty Liver Disease. Am J Chin Med 2018; 46(6):1-18.
10. Enooku K, Nakagawa H, Fujiwara N. Altered serum acylcarnitine profile is associated with the status of nonalcoholic fatty liver disease (NAFLD) and NAFLD-related hepatocellular carcinoma. Sci Rep 2019; 9(1):10663.
11. Simon TG, Kartoun U, Zheng H, Chan AT, Chung RT, Shaw S, Corey KE. Model for end-stage liver disease Na Score predicts incident major cardiovascular events in patients with nonalcoholic fatty liver disease. Hepatol Commun 2017;1(5):429-38.
12. FengShen, Rui-Dan Zheng, Xing-Qiang Sun. Gut microbiota dysbiosis in patients with nonalcoholic fatty liver disease. Hepatobiliary Pancreat Dis Int 2017; 16(4):375-381.
13. Zhao RH, Zheng PY, Liu SM. [Correlation between gut microbiota and behavior symptoms in children with autism spectrum disorder]. Zhongguo dang daierkezazhi = Chinese journal of contemporary pediatrics, 2019; 21(7):663.
14. Cuskin F, Lowe E C, Temple M J, Zhu Y, Cameron EA, Pudlo NA, Porter NT, Urs K, Thompson AJ, Cartmell A, Rogowski A, Hamilton BS, Chen R, Tolbert TJ, Piens K, Bracke D, Vervecken W, Hakki Z, Speciale G, Munōz-Munōz JL, Day A, Peña MJ, McLean R, Suits MD, Boraston AB, Atherly T, Ziemer CJ, Williams SJ, Davies GJ, Abbott DW, Martens EC, Gilbert HJ. Corrigendum: Human gut Bacteroidetes can utilize yeast mannan through a selfish mechanism. Nature 2015; 520(7547):388.
15. Christian M, Gabriele A L, Sabrina D, Francesca T, Leonardo M, Chiara F, Marta M,Arancha H, Alice V, Matthias S, Stefania A, Borja S, Jonathan L, Doyle VW, Rita H, Diego M, Nicola S, Abelardo M, DouweV S. Bifidobacteria exhibit social behavior through carbohydrate resource sharing in the gut. Sci Rep 2015; 5(3):15782.
16. Meiyuan Zhang, Weilan Sun, Minghao Zhou. MicroRNA-27a regulates hepatic lipid metabolism and alleviates NAFLD via repressing FAS and SCD1. Sci Rep 2017, 7(1):14493.
17. Zhu JJ, Cheng ML, Zhou MY, Zhao XK. The effect of p-JAK1/p-STAT3 signaling way in intervening NAFLD by blueberry probiotic serum. Sichuan da xue xue bao. Yi xue ban= Journal of Sichuan University. Medical Science Edition. 2017 Mar 1;48(2):203-9.
18. EL-Shahat A. Toson, Gamal E. Shiha, Asmaa E. Abdelgaleel. Fibrogenic/Angiogenic Linker for Non-Invasive Assessment of Hepatic Fibrosis Staging in Chronic Hepatitis C Among Egyptian Patients. Rev Gastroenterol Mex 2017; 16(6):862.
19. Akin D, Ozmen S, Yilmaz ME. Hyaluronic acid as a new biomarker to differentiate acute kidney injury

from chronic kidney disease. Iran J Kidney Dis 2017;11(6):409.

20. Wang W, Zhao J, Gui W, Sun D, Dai H, Xiao L, Chu H, Du F, Zhu Q, Schnabl B, Huang K. Tauroursodeoxycholic acid inhibits intestinal inflammation and barrier disruption in mice with non-alcoholic fatty liver disease. Br J Pharmacol 2018;175(3):469-84.
21. Ahani H, Attaran S. Therapeutic potential of Seabuckthorn (Hippophae rhamnoides L.) in medical sciences. *Cell Mol Biomed Rep* 2022; 2(1): 22-32. doi: 10.55705/cmbr.2022.330326.1020.
22. Aziziaram Z, Bilal I, Zhong Y, Mahmod AK, Roshandel MR. Protective effects of curcumin against naproxen-induced mitochondrial dysfunction in rat kidney tissue. *Cell Mol Biomed Rep* 2021;1(1):23-32. doi: 10.55705/cmbr.2021.138879.1001.