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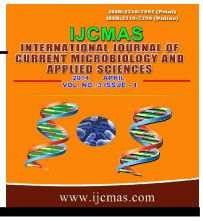

Original Research Article

Effects of Ultra-micro Qiweibaizhusan on Disaccharides Metabolism of Intestinal Microbiotia in Diarrheal Mice with Dysbacteriosis

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A B S T R A C T

To probe into the effects of ultra-micro Qiweibaizhusan on disaccharides metabolism of intestinal microbiotia in diarrheal mice with dysbacteriosis, the diarrheal mice model with dysbacteriosis were constructed with antibiotics mixture composed of Cefradine Capsules and Gentamycin Sulfate injection by intragastric administration. One treatment group′mice were given 100% dose of traditional decoction of Qiweibaizhusan, the other treatment groups′mice were given 50% dose of ultra-micro decoction of Qiweibaishusan, and the normal group′mice and the model group′mice were given with steriled water by intragastric administration. After three days treatment, the intestinal microbiotia were extracted and cultured under aerobic and anaerobic conditions respectively, and their disaccharides metabolism were determined by Biolog Microbial Identification System. After 120 h, the metabolism of D-cellobiose by intestinal microbiotia was the 100% dose of traditional decoction treatment group > the model group > the 50% dose of ultra-micro decoction treatment group > the normal group under aerobic condition, the model group > the 50% dose of ultra-micro decoction treatment group > the 100% dose of traditional decoction treatment group > the normal group under anaerobic condition. And the metabolism of α-D-lactose by intestinal microbiotia was the normal group> the model group > the 100% dose of traditional decoction treatment group > the 50% dose of ultra-micro decoction treatment group under both aerobic and anaerobic conditions. Results indicated that the antibiotics had enhanced the metabolism of D-cellobiose and weaken the the metabolism of α-D-lactose of the microbiotia in diarrheal mice. The metabolism of the two kinds of disaccharides by intestinal microbiotia in mice were gradually changed by the regulatory functions of Qiweibaishusan. The 50% dose of ultra-micro and 100% dose of traditional decoction of Qiweibaishusan had significant differences in regulatory functions of intestinal microflora in diarrheal mice, and had effect on metabolism for D-cellobiose and α-D-lactose of intestinal microflora in mice.

Keywords

Ultra-micro Qiweibaizhusan; intestinal microbiotia; D-cellobiose; α-D-lactose; Chinese medicine

Introduction

There are a large number of microorganisms like bacteria, archaea, and fungi, living in the normal human intestine with their total weight up to 1.0 kg-1.5 kg. Intestinal bacteria and archaea can be respectively divided into fifty genera and

hundreds of species. All of these microorganisms and intestinal environment jointly construct a balanced system (Backhed et al., 2005; Eckburg et al., 2005). The physiological features of intestinal flora are affected directly or indirectly by lots of factors such as growth, reproduction, metabolism, permanent planting, and so on. The hosts affect intestinal flora, and intestinal microbiotia are closely linked with hosts' nutrition, immunity, and metabolism in turn (Round and Mazmanian, 2009).

Cellobiose can be oxidized to lactone by cellobiose dehydrogenase (CDH). CDH is a kind of flavohemoglobin, and this enzyme was compounded by some filamentous fungi which can degrade lignocellulose. CDH is able to reduce multiple substances by using cytochrome C, Fe^{3+}, O_2, and so on as electron acceptor of cellobiose. Compared with other electron acceptors, CDH′s speed of reducing O_2 is rather slow, so it is always called dehydrogenase (Fang Jing, Gao Peiji, 2000; Peng Haizhen, Ren Lihong, 2011). Lactose can′t be fully used until it is hydrolyzed to glucose and galactose by lactase. Once the lactase is low content, poor activity and lacked, the lactose can not be hydrolyzed and it will be fermented by intestinal bacteria to produce a large amount of gas. At the same time, the osmotic pressure of enteric cavity will go up and obstruct its absorption of water to abdominal distension and diarrhea. Antibiotic associated diarrhea is one of diarrhea related to lactase which has large number of reports at present. Antibiotic associated diarrhea was caused because of declining of the fast regeneration of intestinal mucosa and lactase activity by antibiotics. Biolog Microorganism Automatic Analysis System mainly conducts analysis in accordance with microorganisms' utilization rate of carbon source. It can reflect the activity of corresponding key enzyme by measuring microorganisms' capacity to single carbon source in the metabolic pathways of objectives. Microorganisms' specific metabolic capability can be intuitively showed (Cheng Chi et al., 2006; Hu Guilin et al., 2007; Bo Zhongzhong et al., 2009). To understand the specific carbon source metabolism ability of intestinal microflora by using Biolog analysis method under aerobic and anaerobic conditions, the regulation functions of ultra-micro Qiweibaizhusan on the intestinal microbial metabolism will be probed into and its therapeutic mechanism will be elucidate .

Materials and Methods

Animals

24 cleaning grade Kunming mice, half male and half female, body mass 20±2 g, provided by Shanghai Slaccas Laboratory Animal Co., LTD/ Chinese Academy of Sciences, Shanghai Laboratory Animal Center.

Medicine

Ultra-micro Qiweibaizhusan: ginseng (Shanxi Province) 6 g, elecampane (Yunnan Province) 6 g, white poria (Yunnan Province) 10 g, roasted atractylodes macrocephala koidz (Zhejiang Province) 10 g, agastache leaf (Guangdong province) 10 g, the root of kudzu vine (Hunan Province) 10 g, liquorice (Inner Mongolia Province) 3 g. All were purchased from the First Affiliated Hospital of Hunan University of Chinese Medicine. Weighing appropriate medicine according to the above ratio, adding cold water over the medicine level,

and decocting the medicine for twice after half an hour of steeping with big fire until it boils and then with soft fire. The decoction time should not be too long for each time, usually 15-20 minutes. The two times liquid medicine should be mixed to make 100% dose of traditional decoction of Qiweibaizhusan and kept in 4℃ refrigerator.

Ultra-micro medicine was made by smashing the single Chinese herb into ultra-micro powder, and weighing appropriate ultra-micro powder according to the above ratio, adding appropriate boiled water to brew and stir, getting the supernate by low speed centrifugation after cooling, making ultra-micro medicinal slices and decoction of 50% dose, and keeping them in 4℃ refrigerator.

Instruments

Biolog microorganism automatic analyzer (model: E1×808BLG), anaerobic workstation (model: BAETRON-1), bechtop (model: SW-CJ-1B), workplace: Guangdong Ecological Environment and Soil Quality Institute, Guangdong Environmental Sciences and Technology Public Laboratory.

Reagents

Cefradine Capsules (produced by Ouyi Pharmaceutical co., LTD of CSPC, Batch Number: 101005), gentamicin sulphate injection (Shandong Cisen Pharmaceutical Co., Ltd, Batch Number: 100928353). Mixed antibiotics liquor of 62.5 g/L concentration with stroke-physiological saline solution, and kept in 4℃ refrigerator.

Forage

Provided by Animal Experiment Center of Hunan University of Chinese Medicine.

Methods

Grouping the animals

After 2 days of adaptive feeding, 24 mice were divided into four groups namely normal group, model group, 100% dose of traditional Qiweibaizhusan treatment group, and 50% dose of ultra-micro Qiweibaizhusan treatment group. There were 4 groups in total with three males and three females in each group. All of the mice were fed in separate cages.

Modeling method

(Zeng Ao, et al., 2012) Normal group received a gavage of 0.4 mL sterile water for each mouse a time, other groups were given 0.4 mL mixed antibiotics liquor for each mouse a time. It continued for 5 days with twice a day.

Medication and dose

After the success of modeling, intragastric administration was applied twice a day for 3 days, normal group and model group were given sterile water, and other groups were dosed according to mice's clinical equivalent dosage namely 0.16 g/(kg·d) for the 100% dose of traditional Qiweibaizhusan treatment group, and 0.08 g/(kg·d) for 50% dose of ultra-micro Qiweibaizhusan treatment group.

Extracting intestinal contents from mice

Put the mice on bechtop immediately after they were executed by cervical dislocation, made aseptic collection of

contents between jejunum and ileum of each group, mixed up the samples in a aseptic bag full of ice, took it back to laboratory immediately and kept it under -20℃.

Biolog-Eco analysis

This research adopted the Biolog-Eco of 31 kinds of carbon sources to analyze the diversity of enteric microorganism metabolism. The number of carbon source was the same as the references (An Shaoshan et al., 2011). Weighed 5 g intestinal canal content under aseptic condition, put it into a sterilized triangular flask with 45 mL 0.85% NaCl liquor and glass beads, removed the food residue after shaking table vibration for 30 min and 500 r/min centrifugation for 3 min, got 5 mL supernate into 5 sterilized EP tubes, discarded the supernate after 10000 r/min centrifugation for 20 min, and kept the sediment. Added 1 mL 0.85% NaCl sterile water into each EP tube, collected the sediment in a sterilized triangular flask with 45 mL 0.85% NaCl liquor and glass beads after vortex shaking, acquired the microorganisms extracting solution of intestinal canal contents after vibration for 10 min, diluted it to the rate of 1:1000, and used it for Biolog analysis.

Used 8-channel sample injector to add 1:1000 dilute bacterial liquid onto the Biolog - Eco culture plate with each hole 150 μL. Each sample was inoculated two plates. Put the inoculated culture plates into 37 ℃ anaerobic incubator and 37℃ aerobic incubator separately, and used Biolog reading system to read the data at 4h, 12h, 24h, 48h, 60h, 72h, 96h, and 120h of culture. The specific could be referred to Classen's methods (Classen et al., 2003; Liu Feng et al., 2007) .

Statistic analysis

Mice enteric microorganism's metabolism condition of carbon sources could be represented by average absorbance value A, and the computational formula was as follows:

$$A = \sum(C_i - R)/3$$

In the formula, C_i refers to the carbon source holes' difference of absorbance values under 590 nm and 750 nm, R means the A1's absorbance value of Eco plate (Grove et al., 2004).

Used Microsoft Excel 2003 to calculate the data and map, DPS v7.05 to conduct T test, and SPSS19.0 software to conduct principal component analysis. All of the data were the average value of 3 times repetition.

Results and Discussion

Effects of ultra-micro Qiweibaizhusan on D-cellobiose metabolism by intestinal microorganisms in mice

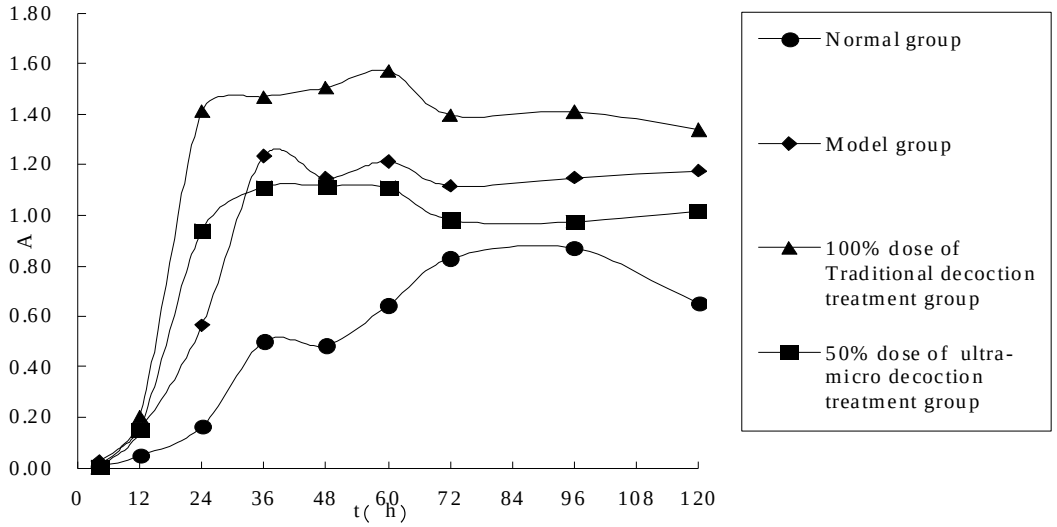
Intestinal microorganisms were inoculated in BIOLOG-ECO plate to culture. When the microorganisms fermented carbon sources to breath, a series of redox reactions and electrons were generated. And TTC (Tetrazolium Violet, 2, 3, 5-Triphenyl Tetrazolium Chloride) was turned from achromatic oxidized form to violet reduced form. Reading system controlled by computers was used to read absorbance value. The larger the absorbance value was, the more the usage of carbon sources was. D-cellobiose is the hydrolysate of cellulose and also cellulose basic structural unit which can be oxidated to lactone by cellobiose dehydrogenase (CDH). Under aerobic culture condition

(Figure 1A), D-cellobiose used by enteric microorganisms in each group increased rapidly especially in the 100% dose of traditional decoction treatment group since the culture was 12 h. During being cultured, enteric microorganisms in the 100% dose of traditional decoction treatment group used the most D-cellobiose. On the contrary, normal group was the least all the way. Till 36 h , the usage of D-cellobiose in model group began to surpass that in the 50% dose of ultra-micro decoction treatment group. The relationship of each group's usage of D-cellobiose from the most to the least is as follows: The 100% dose of traditional decoction treatment group>model group>the 50% dose of ultra-micro decoction treatment group>normal group. It sustained till the end of 120 h . Under anaerobic culture condition (Figure 1B), D-cellobiose used by enteric microorganisms in each group increased rapidly especially in the normal group. It continued till culturing 96 h and then began to stabilize. After being cultured 72 h, enteric microorganisms reduced the usage of D-cellobiose in model group and 100% dose of traditional decoction treatment group rapidly. After being cultured 96 h, enteric microorganisms started the usage of D-cellobiose in each group to stabilize. The relationship from the most to the least was as follows: model group>50% dose of ultra-micro decoction treatment group>100% dose of traditional decoction treatment group >normal group. Figure 1 showed that the usage of D-cellobiose in normal group was the least, while the relationship of other groups was different under aerobic condition and anaerobic condition. It might be connected with the influence of antibiotic and the regulating function of Qiweibaizhusan. It might be caused by different usage situations of aerobic bacteria and anaerobic bacteria for D-cellobiose metabolism in enteric microorganism, which needs further research. In addition, ultra-micro Qiweibaizhusan is rich in cellulose which can produce cellobiose through hydrolysis and be oxidated to lactone by cellobiose dehydrogenase. It might cause the usage of cellobiose by enteric microorganisms.

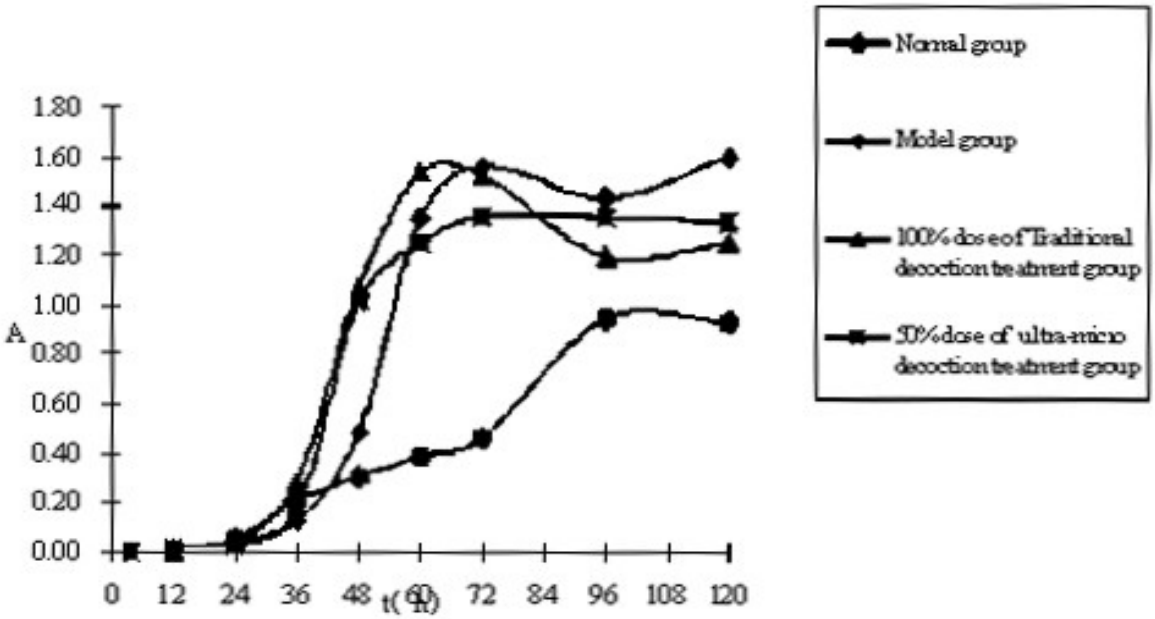
Effects of ultra-micro Qiweibaizhusan on α - D- lactose metabolism by intestinal microorganisms in mice

Lactose couldn't be absorbed directly in small intestine by human body until it was digested by small intestine's lactase.α-Galactosidase (EC 3.2.1.22) was also called melibiase which could hydrolysis α-galactoside. Bacteria, fungi, actinomycetes and yeast can all synthesized galactosidase. The figure 2A showed that enteric microorganisms increased to use α-D- lactose rapidly in each group under aerobic condition since the beginning culture. The usage of α-D- lactose slowed down until the 36 h. And the usage capability of α-D- lactose in normal group was the strongest and that in 50% dose of ultra-micro decoction treatment group was the weakest. After 96 h, the metabolic capability of α-D- lactose in 50% dose of ultra-micro decoction treatment group was weakened rapidly. The figure 2B also showed that enteric microorganisms increased to use α-D- lactose rapidly in normal group after 24 h under anaerobic condition, and weakened after 72 h. Enteric microorganisms strengthened use of α-D- lactose rapidly in the 100% dose

Figure.1 Effects of ultra-micro Qiweibaizhusan on D- cellobiose metabolism by intestinal microorganisms in mice under aerobic condition (A) and under anaerobic condition (B)

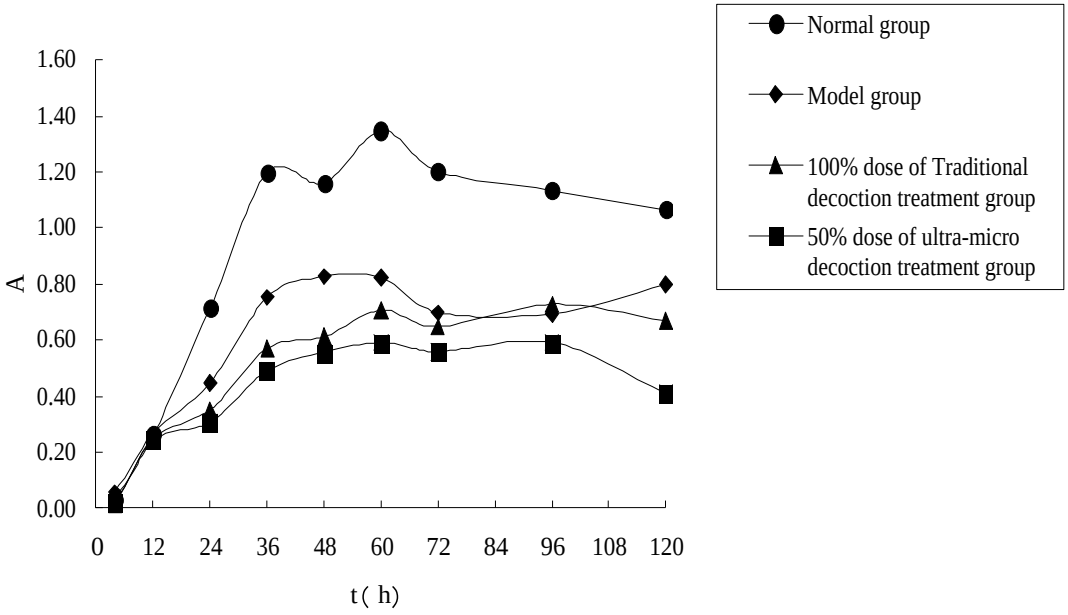


A

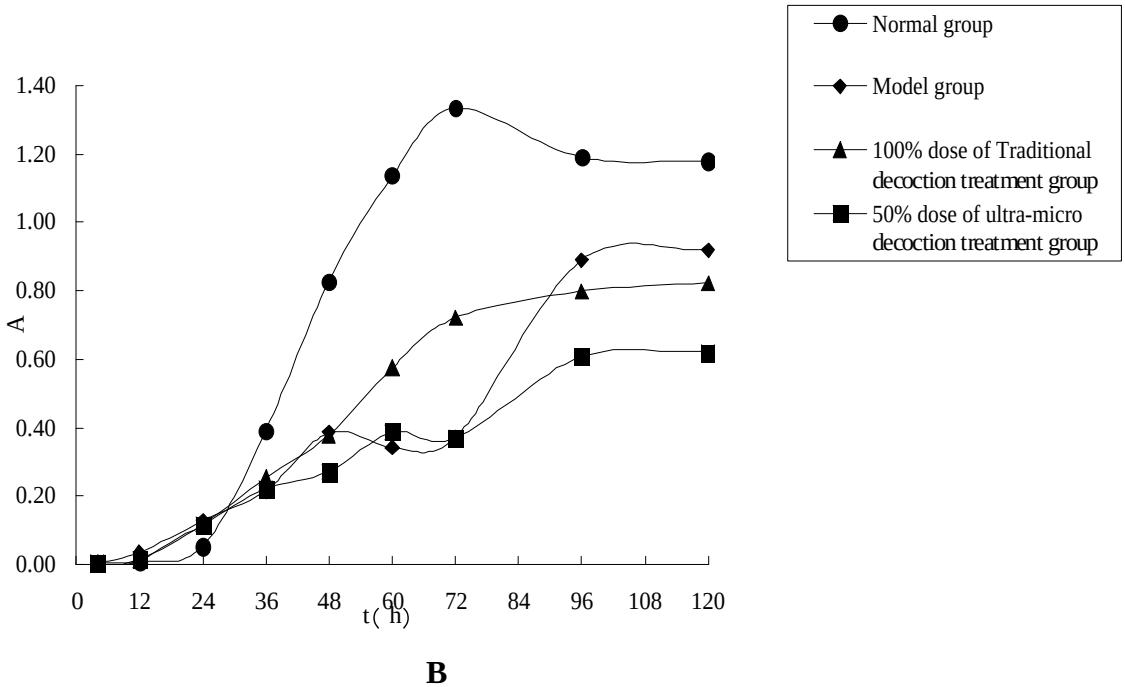


B

Figure.2 Effects of ultra-micro Qiweibaizhusan onα-D- lactose metabolism by intestinal microorganisms in mice under aerobic condition (A) and under anaerobic condition (B)



A



B

Table.1 PCA Factor Loading Matrix of D-cellobiose and α-D-lactose

Culture mode	Group	Carbon source	Factor Load Matrix	
			Component 1	Component 2
Aerobic culture	Normal group	D-cellobiose	0.994	0.107
		α-D lactose	0.997	-0.083
	Model group	D- cellobiose	0.179	0.984
		α-D lactos	-0.677	0.736
	Traditional decoction treatment group	D-cellobiose	0.034	-0.068
		α-D lactose	0.049	-0.003
	50% ultra-micro decoction treatment group	D-cellobiose	0.313	-0.950
		α-D lactose	-0.874	-0.486
Anaerobic culture	Normal group	D-cellobiose	-0.738	0.675
		α-D lactose	-0.971	-0.241
	Model group	D-cellobiose	1.000	-0.007
		α-D lactose	0.997	0.080
	Traditional decoction treatment group	D-cellobiose	0.067	0.998
		α-D lactose	0.405	0.914
	50% ultra-micro decoction treatment group	D-cellobiose	0.810	0.587

of traditional decoction treatment group since 12 h, and started to stabilize from 72 h to the end of 120 h. After 96 h , the usage of α-D- lactose in each group tend toward stability until the end of culture. The relationship was normal group>model group>100% dose of traditional decoction treatment group >50% dose of ultra-micro decoction treatment group. Under aerobic condition and anaerobic condition, metabolism capacity of enteric microorganisms' for α-D- lactose in normal group was the strongest and that in 50% dose of ultra-micro decoction treatment group was the weakest. It turned out that antibiotic molding weakened mice enteric microorganisms' metabolic capability of α-D- lactose, which might be in relationship with antibiotic influences on intestinal lactase's epithelial cells desquamation and enteric microorganisms. After 120 h, metabolic capability of α-D-lactose in model group was stronger than that in all treatment groups, which might be connected with ultra-micro Qiweibaizhusan's recovery effect on intestinal epithelial cells. It showed that the effects of 50% dose of ultra-micro decoction Qiweibaizhusan on intestinal mucosa of mice with dysbacteriosis diarrhea were better than that of 100% does of traditional decoction Qiweibaizhusan (Zhang Hualing, et al., 2013). Therefore, it was able to minish and to eliminate intestinal epithelial cells' influence on lactase activity, causing enteric microorganisms' decreasing synthesis and secretion of lactase. Compared with model group, the utilization capacity of α-D- lactose in treatment groups was weaker. In addition, 50% dose of ultra-micro decoction Qiweibaizhusan was weaker than 100% does of traditional decoction Qiweibaizhusan.

PCA factor loading matrix of D-cellobiose and α-D-lactose on Biolog Eco plates

PCA factor loading could reflect the carbon metabolism differences of enteric microorganism in each group under different conditions. The higher the absolute value was, the bigger the influence of the carbon source would be. Table 1 showed that under aerobic condition, D-cellobiose in normal group contributed more to principal component 1, while D-cellobiose in model group and 50% dose of ultra-micro decoction treatment group contributed more to principal component 2. D-cellobiose in 100% dose of traditional decoction treatment group contributed little to both principal components. Under anaerobic condition, D-cellobiose in model group contributed more to principal component 1, while D-cellobiose in 100% dose of traditional decoction treatment group contributed more to principal component 2. D-cellobiose in normal group and 50% dose of ultra-micro decoction treatment group contributed little to both principal components. Enteric microorganisms in the same group had obvious utilization variance of D-cellobiose under various conditions. Under aerobic condition, α-D-lactose in normal group contributed more to principal component 1, while α-D-lactose in model group, 100% dose of traditional decoction treatment group and 50% dose of ultra-micro decoction treatment group contributed little to both principal components. It indicated that antibiotic modeling affected enteric microorganisms' metabolism capacity of α-D-lactose. Under anaerobic condition, α-D-lactose in normal group, model group, and 50% dose of ultra-micro decoction treatment group contributed more to principal component 1, while α-D-lactose in 100% dose of traditional decoction treatment group contributed more to principal component 2. It indicated that ultra-micro Qiweibaizhusan improved enteric microorganisms to use α-D-lactose. Enteric microorganisms' utilization situation of α-D-lactose in normal group was comparatively consistent under both aerobic and anaerobic conditions, while that in other groups were obvious differences. In model group, 100% dose of traditional decoction treatment group, and 50% dose of ultra-micro decoction treatment group, α-D-lactose's rate of contribution to two principal components under anaerobic condition was higher than that under aerobic condition. It showed that anaerobic bacteria's metabolic capability of α-D-lactose was stronger than aerobic bacteria. The treatment of 50% dose of ultra-micro decoction treatment group was equivalent to that of 100% dose of traditional decoction treatment group (Tan Zhoujin, et al., 2012). However, D-cellobiose and α-D-lactose in the two groups contributed differently to principal components, so their regulatory effects on enteric microorganisms were different.

Normally, intestinal microflora formed a relatively stable and interacting micro-ecosystem which was of great significance to keeping intestinal function, maintaining intestinal mucosal barrier integrity, permanent planting of antagonism resistance microbes, and regulating human body's immunologic function (Zang Jingyuan, Zhang Renhua, 2012). Application of a large amount of broad-spectrum antibiotic would restrain the vast majority of sensitive microbes in intestinal canal, breaking the normal microflora balance of intestinal canal, causing pathogenic bacteria and conditioned pathogen to invade, and bringing about

immunity decline of intestinal canal and digestive dysfunction. Enteric microorganisms were the main completers of various digestions in intestinal canal, and all kinds of enzymes produced by enteric microorganisms played a vital role. Microorganism would generate various kinds of enzymes in the vegetation process such as cellulase, amylase, protease, and so on. In this study, enteric microorganisms' utilization capacity of D-cellobiose in normal group was weaker than that in other groups no matter under aerobic or anaerobic conditions, while its utilization capacity of α-D- lactose in normal group was stronger than others. The reason was that mice's intestinal micro-ecosystem was broken after using antibiotic to conduct gavage, causing the change of its composition and construction. It would affect the synthesis and secretion of cellobiose dehydrogenase and lactase, then led to metabolism change of two kinds of disaccharide.

D- cellobiose could be oxidated to lactone by cellobiose dehydrogenase which was found in white rot, brown rot, and soft-rot fungi containing FAD NRcRd and a heme perssad (Wang Xiguo et al., 2005). In this study, after cultured 96 h , enteric microorganisms metabolic capability of D-cellobiose in model group under aerobic condition was stronger than 50% dose of ultra-micro decoction treatment group and was weaker than 100% dose of traditional decoction treatment group. Enteric microorganisms metabolic capability of D-cellobiose in 50% dose of ultra-micro decoction treatment group was stronger than 100% dose of traditional decoction treatment group and was weaker than model group. Ultra-micro Qiweibaizhusan was full of complicated chemical components and various functions. The effective constituents of ultra-micro decoction and traditional decoction had different dissolution conditions, complex regulatory effects on microorganisms, and apparent different abilities of regulating and controlling aerobic bacteria and anaerobic bacteria. It caused discrepancy of enteric microorganisms' usage of D-cellobiose in two decoction treatment groups under aerobic and anaerobic conditions.

Lactose couldn't be absorbed directly in small intestine by human body until it was digested to galactose and glucose through lactase. Lactase in intestines had two kinds of sources. One part of lactase comes from intestinal epithelial cast-off cells which were released after cells' disintegration and adhere to enteric cavity surface of microvillus. It was the constituent part of microvillus cells' brush border (Zhang Xiaoli, 2010). The other part came from enteric microorganisms, such as bacteria, streptomyces, and yeast (Wang Min et al., 2003). Ultra-micro Qiweibaizhusan has comparatively better recovery effects on intestinal mucosa construction of mice with dysbacteriosis diarrhea, and also cast-off intestinal villi situation (Zhang Hualing et al., 2013). In addition, 50% dose of ultra-micro decoction has better treatment effect than 100% dose of traditional decoction, making activity of lactase which comes from intestinal epithelial cast-off cells strengthened in two treatment groups. The lactase coming from enteric microorganisms may be with antagonistic effects, causing enteric microorganisms' metabolic capability of lactose in two treatment groups weaker than that in model group, and 50% dose of ultra-micro decoction treatment group weaker than traditional decoction treatment group. Human galactosidase synthesized by microorganisms belongs to induced enzyme. When synthesizingα-

galactosidase, different kinds of microorganisms may need various inductors. There are differences between two kinds of ultra-micro Qiweibaizhusan decoction's regulatory effects on enteric microorganisms (Tan Zhoujin et al., 2013). So microorganisms' capacity of synthesizing lactase also may vary, causing enteric microorganisms' different metabolic capabilities of α-D-lactose in two groups

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