Sulfation pattern of fucose branches affects the anti-hyperlipidemic activities of fucosylated chondroitin sulfate

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Abstract

Fucosylated chondroitin sulfates (fCSs) are glycosaminoglycans extracted from sea cucumbers, consisting of chondroitin sulfate E (CSE) backbones and sulfated fucose branches. The biological properties of fCSs could be affected by the sulfation pattern of their fucose branches. In the present study, two fCSs were isolated from sea cucumber *Isostichopus badionotus* (fCS-*Ib*) and *Pearsonothuria graeffei* (fCS-*Pg*). Their monosaccharide compositions were as follow: glucuronic acid (GlcA), N-acetylgalactosamine (GalNAc), fucose (Fuc) and sulfate at similar molar ratio, which was 1.0/0.7/0.9/3.1 for fCS-*Ib* and 1.0/0.8/1.5/2.6 for fCS-*Pg*. The two fCSs have different sulfation patterns on their fucose branches, fCS-*Pg* with 3,4-*O*-disulfation while fCS-*Ib* with 2,4-*O*-disulfation. Their antihyperlipidemic effects were compared using a high-fat high-fructose diet (HFFD)-fed C57BL/6J mice model. Results show that both fCS-*Ib* and fCS-*Pg* had significant effects on lipid profile improvement, liver protection, blood glucose diminution and hepatic glycogen synthesis. Specifically, fCS-*Pg* with 3,4-*O*-disulfation fucose branches was more effective in reduction of blood cholesterol (TC), low density lipoprotein (LDL) and atherogenic index (AI). Our results indicate that both fCSs, especially fCS-*Pg*, could be used as a potential antihyperlipidemic drug.

Key words: Fucosylated chondroitin sulfates, *Isostichopus badionotus*, *Pearsonothuria graeffei*, sulfation pattern, anti-hyperlipidemic

1. Introduction

A great deal of attention is focused on the increasing incidence of hyperlipidemia because of its close connection to the prevalent cardiovascular diseases. Although currently available antihyperlipidemic drugs, such as atoryastatin, lovastatin and fibrates, perform effectively on the prevention of cardiovascular diseases, they have a number of adverse effects (X. Liu, Sun, Zhang, Meng, Xia, Yuan, et al. 2012) such as, hyperuricemia, impotence, gynecomastia, peripheral neuropathy, memory loss, muscle damage, body ache and skin rashes (Bidkar, Ghanwat, Bhujbal, & Dama, 2012)

etc. Moreover, the required long-term treatments for several weeks will further aggravate adverse effects and cause liver damage (Carvalho, 2004). Therefore, it is essential to develop biologically active components from natural materials as safe and effective lipid regulators.

Recently, researchers have paid more attention to marine sulfated polysaccharides for their diverse functions and affluent resources. Sulfated polysaccharides possess a wide range of bioactivities such as anti-tumor, anti-virus, anti-cardiovascular disease, antioxidant and immune adjustment activities (Buyue & Sheehan, 2009; Kiew & Don, 2012; Sheehan & Walke, 2006; M. Y. Wu, Huang, Wen, Gao, He, Li, et al., 2012). However, few reports focused on its effects on lipid metabolism. Recent reports show that sulfated polysaccharides such as *Ulva pertusa* and *Ulva lactuca* possessed antihyperlipidemic activity (Qi, Huang, Liu, Liu, Zhang, & Liu, 2012; Sathivel, Raghavendran, Srinivasan, & Devaki, 2008) which was affected by their molecular weight and sulfate content of sulfated polysaccharides (Qi, Huang, Liu, Liu, Zhang, & Liu, 2012; Yu, 2003). Also, studies show that polysaccharides with more sulfate content exhibited stronger antioxidant, antihyperlipidemic and anticoagulant activities. All the works have proven the sulfate groups in polysaccharides for their activities (Qi, Zhang, et al., 2005; Mestechkina & Shcherbukhin, 2010; Wang, Li, Zheng, Normakhamatov, & Guo, 2007). However, the research of structure-activity relationship of marine sulfated polysaccharides is quite few because of their complicated chemical compositions, broad molecular weights distributions and large numbers of branches, which has limited their further development as marine drugs. Moreover, the complexity of marine sulfated polysaccharides structures also causes difficulties in quality control.

Fucosylated chondroitin sulfates (fCSs) from sea cucumbers have become attractive since their simple, well-defined, repeated structures (S. Chen, Li, Wu, Guo, Liao, Ye, et al., 2013) and potential anticoagulant and antithrombotic properties were studied (Fonseca & Mourao, 2006; Fonseca, Santos, & Mourao, 2009; Mourao, Boisson-Vidal, Tapon-Brethaudiere, Drouet, Bros, & Fischer, 2001; Sheehan & Walke, 2006). fCSs from sea cucumbers *Metriatyla scabra* and *Apostichopus japonicus* were

also reported to have the potential to reduce atherosclerosis and hyperlipoproteinemia risks (H. H. Liu, Ko, & Hu, 2002; X. Liu, et al., 2012). The antihyperlipidemic functions exhibited by fCS could be through following mechanisms: 1) fCSs could inhibit the activity of HMG-CoA reductase (Riessen R. et al., 1999); 2) fCSs could combine with lipids and act as a carrier to participate in the metabolism of cholesterol, therefore accelerating transport and excretion of serum lipids (Wang, Li, Niu, Zhang, & Zhang, 2003); 3) fCSs could act as stimulators of bile acid synthesis and promote the activity of lipoprotein lipase (LPL) through binding to oxidized low density lipoprotein (LDL) by the polysaccharide moiety (Kaplan, M. & Aviram, M., 2000), thus accelerating triglyceride (TG) decomposition into fatty acid, glycerol, and water, and speeding up cholesterol metabolism (Makoveichuk E., Lookene A., Olivecrona G., 1997). Studies showed that the oral administration of fCSs in high fat-fed rats significantly decreased plasma total cholesterol (TC), TG, and low density lipoprotein cholesterol (LDL-C) levels, but increased high density lipoprotein cholesterol (HDL-C) level respectively. However, as previous researches on antihyperlipidemic activity of fCSs were based on few knowledge about their structures, the structure-antihyperlipidemic activity relationship is still unclear. Besides, the health status of livers of those hyperlipidemic mice used in the researches was not assessed.

Two fCSs with well-defined repeating oligosaccharide units were preliminarily identified by 2D nuclear magnetic resonance (NMR): fCS from *Pearsonothuria graeffei* (fCS-Pg) is mainly with 3,4-*O*-sulfated fucose (Fuc3, 4S) branches, while fCS from *Isostichopus badionotus* (fCS-Ib) mainly with 2,4-*O*-sulfated fucose (Fuc2, 4S) branches (S. G. Chen, Xue, Yin, Tang, Yu, & Chai, 2011; N. Wu, Ye, Guo, Liao, Yin, Hu, et al., 2013). The slight difference in the sulfation pattern is the key factor for their differences in the anticoagulant and antithrombotic activities (Chen, Xue et al. 2013). With the abundant information about structures of the two kinds of fCSs, this work is to investigate influences of the fCSs on high-fat high-fructose diet (HFFD) induced hyperlipidemia mice. To the best of knowledge, this is the first study comparing the effects of oral fCSs on the lipid profiles in HFFD induced mice.

2. Materials and methods

2.1. Materials

Two species of sea cucumbers, *Pearsonothuria graeffei* (from Indo-Pacific) and *Isostichopus badionotus* (from Western Atlantic Ocean) were purchased from a local market in Qingdao, Shandong, China.

2.2. Preparation of sea cucumber polysaccharide

Crude sea cucumber polysaccharides were prepared following the previously reported method (Matsuno & Tsushima, 1995). Briefly, sea cucumber body wall (ca. 1 g) was dried, minced, and homogenized. The homogenate was treated with $\text{CH}_3\text{Cl}_3/\text{MeOH}$ (4:1, v/v) to remove lipids before autoclaving at 50°C for 4 h. The resulting residue was digested with 100 mg papain in 30 mL of 0.1 M sodium acetate buffer solution (pH 6.0) (5mM EDTA and 5mM cysteine) at 60°C for 10 h. The digested mixture was centrifuged (2000×g, 15 min, 10 °C) and the polysaccharide in the clear supernatant was precipitated with 1.6mL of 10% aqueous cetylpyridinium chloride solution. After standing at room temperature for 24 h, the mixture was centrifuged (2000×g, 15 min) and the precipitated polysaccharide was collected and redissolved in 10mL of 2M NaCl:ethanol (100:15, v/v) before further precipitation with 20mL of 95% ethanol. After standing at 4°C for 24 h, the precipitate was collected by centrifugation (2000 × g, 15 min). Then, the precipitate was dissolved in water and dialyzed against distilled water (with two exchanges). The polysaccharide solution was lyophilized before analysis.

The crude polysaccharide solution was fractionated by anion-exchange chromatography on a DEAE-cellulose column (1.6×17cm, Whatman, Brentford, England) with elution by a linear gradient of 0–1.4 M NaCl in 1000 min at a flow rate of 0.5 mL/min. Carbohydrate fractions were collected every 10 minutes with a test tube. Polysaccharide content was determined by improved phenol/sulfuric assay (S. G. Chen, Xue, Yin, Tang, Yu, & Chai, 2011). The respective components in test tubes were further determined by high performance liquid chromatography (HPLC) with a TSK gel PWXL-4000 column (Tosoh Bioscience, Tokyo, Japan). The polysaccharide

solution collected and dialyzed against distilled water was lyophilized.

2.3. *Animals and design*

Fifty-six C57BL/6J mice, male, weighting from 18 to 22 g, were purchased from the Animal Lab Center of Zhejiang Chinese Medical University (Certificate No. SCXK (Hu) 2007-2005, China). The animals were housed in stainless steel cages at room temperature (25 ± 2 °C) with a 12-h light/dark cycle. The animals were fed with a commercial mice chow for 3 days to acclimatize to animal facilities. Then, animals were weighed and randomly divided into seven groups of 8 mice; Group 1, normal control; Group 2, hyperlipidemic control; Group 3, standard drug (inositol nicotinate, 500 mg/kg), positive control; Group 4, 100 mg/kg fCS-*Ib*; Group 5, 200 mg/kg fCS-*Ib*; Group 6, 100 mg/kg fCS-*Pg*; Group 7, 200 mg/kg fCS-*Pg*. Inositol niacinate is effective in modifying lipid profiles to treat hyperlipidemia, thus it was chosen as a positive drug in this study. After the acclimation ended, Group 1 continued to be provided with the common commercial mice chow and others were fed with the HFFD for 36 days. At the same time, Groups 3 to 7 were given different doses of inositol nicotinate, fCS-*Ib* and fCS-*Pg* by oral administration for 36 days. The mice were allowed free access to food and water during the experimental period. The HFFD consisted of 1% cholesterol, 15% lard, 25% fructose and 59% commercial chow. The food consumption and weight gain were measured every 4 days.

2.4. *Oral glucose tolerance test*

On day 28, the oral glucose tolerance test was performed in overnight (12 h) fasted animals. Glucose solution (2 g/kg BW) was administered orally at 0, 30, 60 and 90 min respectively. Blood was drawn from a tail vein to measure the blood glucose level with one touch glucometer (Accu-chek Performa).

2.5. *Measurement of serum lipids*

At the end of the experimental period (36 days), the mice were starved for at least 12 h, weighed and anesthetized. Blood samples were collected from the eyeballs

to measure the serum TC, TG, HDL-C and LDL-C levels. Serum TC (Deeg & Ziegenhorn, 1983), TG (Nagele, Hagele, Sauer, Wiedemann, Lehmann, Wahlefeld, et al., 1984) and HDL-C (Allain, Poon, Chan, Richmond, & Fu, 1974) were measured enzymatically and LDL-C levels were calculated from the Friedewald formula (Friedewald, Levy, & Fredrickson, 1972).

The concentrations of glutamate oxaloacetate (GOT) were measured using a commercial kit (Sigma Chemical Co., MO, USA) based on the method of Reitman and Frankel (1957) (Reitman & Frankel, 1957).

2.6. Determination of liver weight and hepatic glycogen

On day 36, after 24h fasting, mice were killed by decapitation, and their livers were quickly removed and weighed. Hepatic glycogen contents were measured based on the anthrone colorimetric method (Carroll, Longley, & Roe, 1956).

2.7. Statistical analysis

All data are presented as the mean $\pm$ SE. The data was evaluated by one-way ANOVA using a Statistical Package for Social Sciences software program (SPSS Inc., Chicago, IL, USA) and the differences between the means were assessed using Duncan's multiple range test. Statistical significance was considered at $P < 0.05$.

3. Results and discussion

3.1. Chemical composition of fCS-Ib and fCS-Pg

fCS-Ib and fCS-Pg were isolated as previously reported (S. G. Chen, Xue, Yin, Tang, Yu, & Chai, 2011) and purified by anion-exchange chromatography. Monosaccharide composition analysis of the two fCSs indicated they contained glucuronic acid (GlcA), N-acetylgalactosamine (GalNAc), fucose (Fuc) and sulfate at similar molar ratio, which was 1.0/0.7/0.9/3.1 for fCS-Ib and 1.0/0.8/1.5/2.6 for fCS-Pg (Table 1). The average molecular weights of fCS-Ib and fCS-Pg were 109 and

73 kDa respectively. According to the previous detailed analyses of the two fCSs by 2D NMR (Chen et al. 2013), we can conclude that both fCS-*Ib* and fCS-*Pg* contain a standard chondroitin sulfate E (CSE) backbone. The major difference is their sulfation pattern of fucose branches: fCS-*Ib* has a 2,4-*O*-sulfated fucose branch, while fCS-*Pg* has a 3,4-*O*-sulfated fucose as its main branch (Fig. 1).

Figure 1. The main structures of the sulfated polysaccharides from sea cucumbers. (a) fCS-*Ib*; (b) fCS-*Pg*.

Table 1. Chemical composition of fCS-*Ib* and fCS-*Pg*

Sample	MW(kDa)	Ratio*			
		GlcA	GalNAc	Fuc	Sulfate
fCS- <i>Pg</i>	73	1.0	0.8	1.5	2.6
fCS- <i>Ib</i>	109	1.0	0.7	0.9	3.1

*The molar ratio of sugar and sulfate were compared by defining the GlcA content as 1 molar.

3.2. Effects of fCSs on the body weight of mice

Mice in all groups gained body weight (Table 2). Both fCS-*Ib* and fCS-*Pg* treated groups showed dose dependent relationship in lowering the weight gain. fCS-*Ib* inserted more obvious effect as weight gain in Group 4 (100 mg/kg fCS-*Ib*) and Group 5 (200 mg/kg fCS-*Ib*) was obviously lower than that of Group 2 (the hyperlipidemic group), Group 6 and 7 (the fCS-*Pg* groups treated groups). Furthermore, Group 5 (200 mg/kg fCS-*Ib*) showed the least weight gain among all the

sample groups, indicating that fCS-*Ib* at 200 mg/kg helped to curb the body weight increase effectively.

Table 2. Effects of inositol nicotinate, fCS-*Ib* and fCS-*Pg* on body weight in mice.

Group	Dose (mg/kg)	Initial Wt (g)	Final Wt (g)	Gain Wt (g)
1 Normal	-	21.48±0.38	26.58±0.75	5.10±1.03
2 Hyperlipidemia	-	21.88±0.30	27.73±0.31	5.25±0.50
3 Inositol niacinate	500	21.08±0.33	22.86±0.44* [#]	1.79±0.39* [#]
4 fCS- <i>Ib</i>	100	21.33±0.72	24.99±0.70	3.66±1.12
5	200	21.41±0.48	23.55±0.61* [#]	2.14±0.79* [#]
6 fCS- <i>Pg</i>	100	21.05±0.61	25.53±5.29	4.24±1.19
7	200	21.43±0.70	25.08±0.63	4.14±1.09

3.3. Effects of fCSs on serum TC, TG, HDL-C, LDL-C and AI in HFFD induced hyperlipidemic mice

As shown in Table 3, serum TC, TG and LDL-C levels increased significantly and serum HDL-C level decreased significantly in Group2 (the hyperlipidemic group) compared with those in Group 1 (the control group), indicating the model was successfully set.

Among all of the fCSs treated groups, the TC, TG, LDL-C levels decreased significantly while the HDL-C level increased compared to the hyperlipidemic group. The fCS-*Ib* treated groups showed greater dose-dependent effects on lowering TG compared with the fCS-*Pg* treated groups. Compared with the hyperlipidemic group, TG level dropped 55.68% in Group 5 (200 mg/kg fCS-*Ib*) ($P < 0.05$) and 35.22% in Group 7 (200 mg/kg fCS-*Pg*) ($P < 0.05$). As for the HDL-C level, both fCSs treated groups increased HDL-C level with dose dependent relationship and their effects on HDL-C elevation were even greater than that of the positive control group (inositol niacinate). In Group 5 (200 mg/kg fCS-*Ib*) and 7 (200 mg/kg fCS-*Pg*), the HDL-C level increased 18.63% ($P < 0.05$) and 16.35% ($P < 0.05$), which reflected similar

HDL-C elevation function of both fCSs. As for the function of lowering TC and LDL-C levels, both fCSs showed better effects at low dosage (100 mg/kg) rather than high dosage. Compared with the hyperlipidemic group, TC and LDL-C levels were reduced 53.43% and 65.42% in Group 6 (100 mg/kg fCS-*Pg*), while in Group 4 (100 mg/kg fCS-*Ib*) they were reduced 49.6% and 57% respectively.

Hyperlipidemia is considered to be the leading risk factor for atherosclerosis. Abnormal increase in serum TC, TG and LDL-C levels refer to high risk of atherosclerosis. Since atherogenic index (AI) is an important index reflecting atherosclerosis and coronary heart disease risks, the higher AI value reflects higher risk of atherosclerosis. Compared with the hyperlipidemic group, both fCSs treated groups showed significant effects on lowering AI, and such effects were even more obvious than that of the positive control group (inositol niacinate). Especially, Group 6 (100 mg/kg fCS-*Pg*) showed the lowest AI value for about 0.22 ± 0.02 .

High levels of TG, TC and LDL-C would increase blood viscosity and raise the risk for cardiovascular diseases. TC and TG are transported within the body through different kinds of lipoproteins. HDL carries TC from surrounding tissues to liver and then synthesizes bile acid to remove redundant TC through the Reverse Cholesterol Transport, thus preventing TC from gathering on blood vessel wall (Asztalos & Schaefer, 2003). Conversely, LDL is the major carrier of TC towards tissues. Excessive LDL can migrate to the endarterium and thus having the potential to cause atherogenic effects (Kitamura, Iso, Naito, Iida, Konishi, Folsom, et al., 1994). Therefore, increasing serum HDL and reducing serum LDL level can reverse the atherosclerotic pathologic process. According to the results above, fCS-*Ib* and fCS-*Pg* showed marked therapeutic properties on decreasing serum TC, TG, LDL-C concentrations and increasing serum HDL-C concentration in hyperlipidemic mice. The results were also in accordance with previous research on the antihyperlipidemia activity of fCSs from *Apostichopus japonicas* (X. Liu, et al., 2012) and *Metriatyla scabra* (H. H. Liu, Ko, & Hu, 2002).

Table 3. Effects of inositol nicotinate, fCS-*Ib* and fCS-*Pg* on serum lipid profiles in hyperlipidemia mice.

Group		Dose	TC	TG	HDL-C	LDL-C	AI
		(mg/kg)	(mmol/L)	(mmol/L)	(mmol/L)	(mmol/L)	
1	Normal	-	3.83±0.13	1.33±0.13	2.94±0.12	0.36±0.03	0.31±0.02
2	Hyperlipidemia	-	7.58±0.38 [#]	1.76±0.22 [#]	2.63±0.13 [#]	1.07±0.10 [#]	1.91±0.25 [#]
3	Inositol niacinate	500	3.74±0.16*	0.71±0.09* [#]	2.70±0.11*	0.35±0.02*	0.39±0.02*
4	fCS- <i>Ib</i>	100	3.82±0.31*	0.87±0.08* [#]	2.82±0.29*	0.46±0.04*	0.36±0.04*
5		200	4.04±0.13*	0.78±0.10* [#]	3.12±0.08*	0.47±0.03*	0.30±0.04*
6	fCS- <i>Pg</i>	100	3.53±0.19*	1.23±0.13*	2.88±0.13*	0.37±0.02*	0.22±0.02*
7		200	3.89±0.18*	1.14±0.11*	3.06±0.12*	0.39±0.03	0.27±0.03*

*P < 0.05: compared to hyperlipidemia control group. [#] P < 0.05: compared to normal control group.

According to the previous research, antihyperlipidemic mechanism of polysaccharides may be due to the bile acid sequestrant mechanism, in which polysaccharides act as stimulators of bile acid synthesis (Qi, Huang, Liu, Liu, Zhang, & Liu, 2012). The bile acid pool remains essentially constant by reabsorbing most of the bile acids in small intestine and returning to liver. Bile acid sequestering resins in small intestine interrupt the enterohepatic circulation and increase the fecal excretion of bile acids so that fewer bile acids would return to liver. The loss of bile acids is compensated by the oxidation of more hepatic cholesterol, the only precursor to bile acids, and stimulates the synthesis of bile acids, thereby decreasing the total blood cholesterol level (X. Liu, et al., 2012; Qi, Huang, Liu, Liu, Zhang, & Liu, 2012). Also, the activity of reducing TG may be also related to the lipoprotein lipase (LPL) mechanism (Kwak, Kyung, Kim, Cho, & Rhee, 2010). It is known that LPL is a key atheroprotective enzyme in regulating plasma TG levels by removing TG-rich lipoproteins from circulating plasma (Kiew & Don, 2012). Therefore, fCSs lowered blood TC and TG levels mainly by elevating HDL-C level, reducing LDL-C level, acting as the bile acid synthesis stimulator and by promoting LPL activity.

Since the two fCSs have similar composition and molecular weight, the only difference was the sulfation of their fucose branches. Thus, the antihyperlipidemic activities of fCS-*Ib* and fCS-*Pg* may be related to the sulfation pattern of their fucose branches. Taking all of effects of fCSs on the lipid profile into consideration, fCS-*Pg* exhibited obvious elevation on HDL-C level and greater effects on TC, LDL-C and AI reduction than fCS-*Ib*. Therefore, the 3,4-*O*-disulfation of fucose branch is important for the antihyperlipidemic effects of fCSs. However, low dosage of fCS-*Pg* is recommended possibly because of some side effects of overdosage at 200mg/kg, such as bleeding, which have been reported in fCSs from sea cucumber *L.grease* (Fonseca RJC, et al., 2010).

3.4. Effects of fCS-*Ib* and fCS-*Pg* on liver weight and GOT concentration

Although anti-hyperlipidemic drugs could significantly regulate blood lipid profile, they also insert adverse side effects on liver. Therefore, based on the improvement of regulating blood lipid of fCS-*Ib* and fCS-*Pg*, we further investigated their effects on liver weight and GOT concentration to see whether fCSs could protect liver or not. After 36 days of feeding, cholesterol supplementation resulted in hepatomegaly, as indicated by the significant increase of liver weight in Group 2 (the hyperlipidemic group) compared with Group 1 (the control group) (Table 4). Both fCSs decreased liver weight with dose dependent relationships. The liver weight was significantly decreased by 11.84% (100 mg/kg fCS-*Ib*) ($P < 0.05$), 25.65% (200 mg/kg fCS-*Ib*) ($P < 0.05$), 19.74% (100 mg/kg fCS-*Pg*) ($P < 0.05$) and 27.63% (200 mg/kg fCS-*Pg*) ($P < 0.05$) respectively. This indicated that fCS-*Pg* inserted better effects on liver weight reduction compared with fCS-*Ib*.

Table 4 Effects of inositol nicotinate, fCS-*Ib* and fCS-*Pg* on liver weight and glutamic-oxaloacetic transaminase in hyperlipidemia mice.

Group		Dose (mg/kg)	Liver (g)	GOT(Units/L)
1	Normal	-	1.37±0.09	130.05±36.67

2	Hyperlipidemia	-	1.52±0.04 [#]	205.38±1.82 [#]
3	Inositol niacinate	500	0.86±0.03 ^{*#}	102.11±11.21 ^{*#}
4	fCS-Ib	100	1.34±0.05 [*]	149.56±10.34 [*]
5		200	1.13±0.04 [*]	159.16±3.09 [*]
6	fCS-Pg	100	1.22±0.05 [*]	125.85±29.11 [*]
7		200	1.10±0.06 [*]	158.36±10.23 [*]

* P < 0.05: compared to hyperlipidemia control group. [#] P < 0.05: compared to normal control group.

GOT mainly exists in the mitochondria of liver cells. When serious necrosis or damage appears in liver, the concentration of GOT in serum will increase. Hence, the increasing level of GOT is an important indicator of liver damage and many kinds of liver diseases such as viral hepatitis, liver cirrhosis, liver cancer, fatty liver and alcoholic hepatitis, etc. (Al Kanhal, Ahmad, Al Othman, Arif, Al Orf, & Al Murshed, 2002; Ban, Rico, Um, & Kang, 2012). The significant increase in GOT activity in the hyperlipidemic group compared with the normal control group indicated that long period of HFFD caused pathological changes of liver tissues. Compared with the hyperlipidemic group, GOT concentration dropped by 27.18% (100 mg/kg fCS-Ib) ($P < 0.05$), 22.50% (200 mg/kg fCS-Ib) ($P < 0.05$), 38.72% (100 mg/kg fCS-Pg) ($P < 0.05$) and 22.89% (200 mg/kg fCS-Pg) ($P < 0.05$) respectively. Both fCSs could effectively lower the GOT activity while fCS-Pg was more effective. However, the results also suggested that low dosage fCSs groups (100 mg/kg) served better effects on GOT diminution than the high dosage ones. This may possibly be contributed to the overdosage effects at 200 mg/kg such as mild bleeding, which was also in accordance with some previous studies (Fonseca RJC, et al., 2010). Taking the results of liver weight gain and GOT activity altogether, fCS-Pg with 3,4-*O*-sulfated fucose branch was more effective in liver protection activity compared with fCS-Ib.

3.5. Effects of fCS-Ib and fCS-Pg on blood glucose level and hepatic glycogen content in hyperlipidemia mice

Apart from high fat, the feeding also contained 25% fructose, suggesting that long-term feeding might induce diabetic mice (Kazumi, Odaka, Hozumi, Ishida, Amano, & Yoshino, 1997) and exert adverse effects on blood glucose level of mice. Hence, the blood glucose levels of all groups were tested. The blood glucose level of the hyperlipidemic group significantly increased compared with the normal group, showing that these hyperlipidemia mice were also diabetic. As figure 2 shows, the blood glucose in all groups reached the maximum level 30 min after the oral glucose challenge. All fCSs fed groups significantly suppressed the increase of blood glucose after 30 min, and reached higher levels than their initial at 60 min. This suppression effect on the blood glucose level would persist until the blood glucose level reached the initial level. Among the fCSs treated groups, the high dosage treated mice showed similar potent glucose tolerance as the low dosage ones. Also, fCS-*Ib* and fCS-*Pg* owning different sulfate patterns inserted similar property in regulating glucose tolerance.

Figure 2. Effect of inositol nicotinate, fCS-*Ib* and fCS-*Pg* on blood glucose levels in hyperlipidemia mice. Values are expressed as means±S.D. (n=10 per group).

*P < 0.05: compared to hyperlipidemia control group; #P < 0.05: compared to normal control group

Figure 3. Effect of fCS-*Ib* and fCS-*Pg* on hepatic glycogen in hyperlipidemia mice.

* $P < 0.05$: compared to hyperlipidemia control group; # $P < 0.05$: compared to normal control group.

Blood glucose level in body decreases mainly through promoting hepatic glycogen synthesis, glucose utilization and inhibiting sugar dysplasia. Thus, the ability to stimulate glycogen synthesis is of great importance to reduce the blood glucose level. In Figure 3, the hyperlipidemic group showed great loss in hepatic glycogen, which suggested that normal synthesis of hepatic glycogen had been disturbed due to the HFFD, corresponding with its considerable increase of blood glucose level. Compared with the hyperlipidemic group, hepatic glycogen contents increased by 76.61% (100 mg/kg fCS-*Ib*) ($P < 0.05$), 66.69% (200 mg/kg fCS-*Ib*) ($P < 0.05$), 62.53% (100 mg/kg fCS-*Pg*) ($P < 0.05$) and 45.36% (200 mg/kg fCS-*Pg*) ($P < 0.05$) respectively. Based on these results, fCS-*Ib* was more effective than fCS-*Pg* in stimulating hepatic glycogen synthesis ($P < 0.05$). Taking the results of blood glucose level and hepatic glycogen synthesis altogether, fCS-*Ib* showed more potent effects on glucose regulation than fCS-*Pg*, especially at low dosage.

4. Conclusion

Instead of streptozotocin-induced diabetic mice (Zhu, Nie, Li, Lin, Xing, Li, et al., 2013), cholesterol and fructose-rich diet was used in the present study. Although the main aim was to induce hyperlipidemic mice, diabetic mice were also obtained. The characteristic dysglycemia of HFFD induced hyperlipidemia mice with damaged glucose tolerance and decreased hepatic glycogen content. This means that hyperlipidemia may further develop into diabetes possibly. Using this mice model, we found that both fCS-*Ib* and fCS-*Pg* regulated blood glucose level and improved lipid profile obviously. Specifically, fCS-*Ib* showed better effects on blood glucose reduction and hepatic glycogen synthesis. fCS-*Pg* with 3,4-O-disulfation fucose branch exhibited stronger antihyperlipidemic activity than fCS-*Ib* with 2,4-O-disulfation through significantly lowering serum TG, TC, LDL-C, AI, liver weight and GOT activity and increasing HDL-C. Further study on blood glucose level and hepatic glycogen content indicated that hyperlipidemia may further develop into diabetes and both fCSs may insert anti-hyperglycemia activity. However, further research is needed to determine this effect in the future.

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Abbreviations

AI, atherosclerosis index; CSE, standard chondroitin sulfate E; fCS, fucosylated chondroitin sulfate; fCS-*Ib*, fucosylated chondroitin sulfate from *Isostichopus badionotus*; fCS-*Pg*, fucosylated chondroitin sulfate from *Pearsonothuria graeffei*; Fuc, fucose; GlcA, glucuronic acid; GalNAc, N-acetylgalactosamine; GOT, glutamic-oxaloacetic transaminase; HDL, high density lipoprotein; HDL-C, high density lipoprotein cholesterol; HPLC, high performance liquid chromatography; LDL, low density lipoprotein; LDL-C, low density lipoprotein cholesterol; LPL, lipoprotein lipase; TC, total cholesterol; TG, triglyceride.

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