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Antihyperlipidemic activity of high sulfate content derivative of polysaccharide extracted from *Ulva pertusa* (Chlorophyta)

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ABSTRACT

In this study, high sulfate content ulvan (HU) was prepared with sulfur trioxide/*N*,*N*-dimethylformamide (SO_3–DMF) in formamide, and the antihyperlipidemic activity of ulvan and HU in mice was determined. Obvious differences in antihyperlipidemic activity between natural ulvan and HU were observed, moreover, the antihyperlipidemic activity of HU-fed 250 mg/kg was the strongest, compared to natural ulvan fed group, triglyceride (TG) and low density lipoprotein cholesterol (LDL-C) concentrations were significantly decreased 28.1% ($P < 0.05$) and 28.4% ($P < 0.01$), respectively. It was likely that the sulfate content had significant effect on the antihyperlipidemic activity. On the other hand, the results proved that the antihyperlipidemic activity was not concentration dependent for HU-fed mice.

1. Introduction

Recent researches have demonstrated the relationship between plasma lipid levels and development of atherosclerosis (Brown, 1994). A decrease of serum high density lipoprotein cholesterol (HDL-cholesterol) and increments of low density lipoprotein cholesterol (LDL-cholesterol), and triglyceride (TG) were considered to be significant risk factors (Asztalos & Schaefer, 2003). Many new classes of hypolipidemic agents have been widely used for the improvement of hyperlipidemia associated with atherosclerosis during the past decade (Dujovne & Harris, 1989; Illingworth, 1988; Roth, Sliskovic, & Trivedi, 1989).

The most commonly used lipid regulators at the present time are lovastatin (Statin), gemfibrozil (Fibrates). However, recent reports of undesirable side effects (myopathy) of some "super statins" indicate that the scope of improving the potency of this class of drugs may be modest (Graham et al., 2004). Thus, it is essential to develop and utilize effective and natural lipid regulators so that they can protect the human body from hyperlipidemia. According to reports, chitosan, chitosan derivatives and coumarin bisindole derivatives all showed antihyperlipidemic activity (Kul-Lee, Un-Kim, & Hoe-Kim, 1999; Nauss, Thompson, & Nagyvary, 1983; Sashidhata, Kumar, Kumar, Srivastava, & Puri, 2010; Sugano et al., 1980).

The green alga, *Ulva pertusa*, is an important food source in many parts of the world. *U. pertusa* is nutritious with low calorie, abundant vitamins, trace elements and dietary fibers (Lahaye & Jegon, 1993). Moreover, it has been used as a drug in traditional Chinese medicine for hyperlipidemia, sunstroke and urinary diseases. The polysaccharide extracted from *U. pertusa* is a group of sulfated heteropolysaccharides and the main disaccharide units are [β-D-GlcpA-(1 → 4)-α-L-Rhap3s] and [α-L-Idop A-(1 → 4)-α-L-Rhap3s] (Yu, Zhang, et al., 2003). For simplicity, the sulfated polysaccharide is referred to as ulvan (U) in this paper.

Algal sulfated polysaccharides have been reported to possess diverse biological activity of potential medicinal value, such as anticoagulant, antitumor, anti-inflammatory, antiviral, antihyperlipidemic and antioxidant activity (Feldman, Reynaldi, Stortz, Cerezo, & Damonte, 1999; Qi, Zhao, et al., 2005; Sathivel, Raghavendran, Srinivasan, & Devaki, 2008). The activity of polysaccharide depends on several structural parameters such as the degree of sulfation (DS), the molecular weight, the sulfation position, type of sugar, and glycosidic branching (Melo, Feitosa, Freitas, & de Paula, 2002; Qi, Zhang, et al., 2005; Yu, Liu, Zhou, Zhang, & Li, 2003). Thus, chemical modifications of polysaccharides provided an opportunity to obtain new agents with possible therapeutic

Abbreviations: U, ulvan; HU, high sulfate content ulvan; SO_3–DMF, sulfur trioxide/*N*,*N*-dimethylformamide; TC, total cholesterol; TG, triglyceride; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol.

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uses (Baldwin & Kiick, 2010; Franz & Alban, 1995). In our previous study, we investigated antioxidant activity of different sulfate content ulvans, and found that high sulfated ulvans exhibited stronger antioxidant activity than that unmodified ulvan (Qi, Zhang, et al., 2005). Yu et al. reported that polysaccharide extracted from *U. pertusa* exhibited the antihyperlipidemic actions, moreover, effects on lipid metabolism were modified when it was degraded into low molecular weights fractions without the changes of chemical composition and structure (Yu, Liu, et al., 2003). The evidence had proved that chemical modifications of polysaccharides provided a probability to obtain new antihyperlipidemic agents with possible use. In the present study, high sulfate content ulvan derivative was prepared and their antihyperlipidemic activities were determined. To our knowledge, the antihyperlipidemic activity of high sulfate content ulvan derivative had not been reported.

2. Materials and methods

2.1. Materials

U. pertusa was collected on the coast of Qingdao, China. The algae were washed, air dried and kept in plastic bags at room temperature before use. All reagents were of analytical grade. Dialysis membranes were produced by Spectrum Co., and molecular weight was cut off at 3600 Da and 10,000 Da. All other chemicals and reagents, unless specified otherwise, were not purified, dried or pretreated.

2.2. Preparation of natural ulvan (U)

Two hundred grams dry algae were cut roughly and autoclaved in 8000 mL water at 125 °C for 4 h. The hot aqueous solution was separated by successive filtration with gauze and siliceous earth. The solution was dialyzed against tap water for 48 h and against distilled water for 48 h using 10,000 Da Mw cutoff dialysis membranes, and then concentrated to about 2000 mL under reduced pressure. The polysaccharides were precipitated by the addition of 8000 mL of 95% (v/v) ethanol. The resultant precipitate was washed three times with dry ethanol, and then dried at 80 °C (mean yield, 22.5%).

2.3. Preparation of high sulfate content ulvans (HUs)

The sulfation agent, SO_3–DMF, was obtained by dropping 50 mL of chlorosulfonic acid ($HClSO_3$) into 300 mL of *N*,*N*-dimethylformamide (DMF) under cooling in an ice-water bath. Dry ulvan (2 g) was added to 80 mL of formamide (FA), and the mixture was stirred at different temperatures (60 °C) for 30 min in order to disperse it into solvent. Then SO_3–DMF reagent (25 mL) was added. After 4 h, the mixture was cooled to room temperature by an ice bath, neutralized with 2 M NaOH solution, and precipitated with 85% ethanol for 24 h. The precipitate was filtered off and washed three times with ethanol, and then was dissolved in 100 mL distilled water. The solution was dialyzed against tap water for 48 h and distilled water for 48 h using 3600 Da Mw cutoff dialysis membranes. The resultant was concentrated and lyophilized to give high sulfate content ulvan (Yuan et al., 2005).

2.4. Analytical methods

Sulfate content was determined by using the traditional method of barium chloride–gelatin (Kawai, Seno, & Anno, 1969). FTIR spectra were measured by Nicolet Magna-Avatar 360 with KBr disks. ^{13}C NMR spectra were recorded on a JNM-ECP600 spectrometer in D_2O solvent.

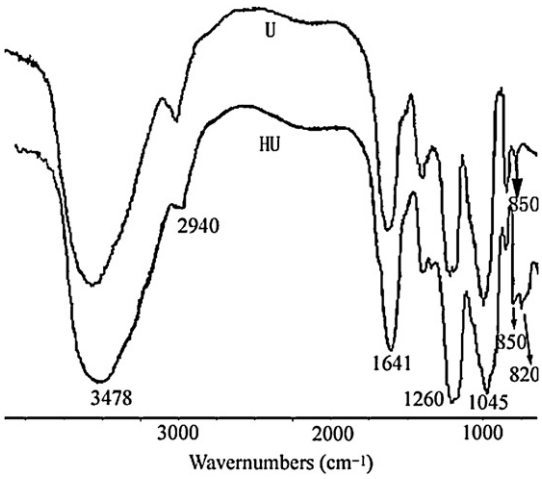

Fig. 1. Infrared absorption spectrum of the U and HU.

2.5. Animals and experimental design

Eighty-four Kunming mice, male/female, weighing from 18 to 22 g, were obtained from the Animal Lab Center of Shandong University (number of animal license SCKX (Lu) 20030004) (China). The animals were housed in stainless steel cages at room temperature (25 ± 2 °C) and 12 h light cycle. The current study protocol was approved by Ethics Committee of Weifang Medical University for animal studies. 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 12 mice. Group 1 was normal control while group 2 served as hyperlipidemic control and group 3 had animals treated with ulvan (250 mg/kg body weight). Groups 4–6 received HU in doses of 125, 250 and 500 mg/kg whereas group 7 had the standard drug (inositol nicotinate, 500 mg/kg) treated animals that served as positive control. After the period of acclimation ended, group 1 continued to be provided with the common commercial mice chow and others were fed with a cholesterol-rich diet for 21 days. At the same time, groups 3–7 were given different dose of U, HU and inositol nicotinate by oral administration for 21 days.

The composition of cholesterol-rich diet was 2.0% cholesterol, 8.0% lard, 0.3% sodium cholic acid and 89.7% commercial chow. Animals had free access to water and food *ad libitum*. At the end of the experimental period (21 days), the mice were withheld food for at least 12 h, weighed and blood samples were collected from the eyeballs to measure the serum TC, TG, HDL-C and LDL-C levels. Serum TC (Dceg & Ziegenhorn, 1983), TG (Nagele, Hagele, & Sauer, 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).

2.6. Statistical analysis

All results were compared using the unpaired Student's *t*-test and values expressed as means ± SD. A probability of $P < 0.05$ and $P < 0.01$ was considered as significant.

3. Results and discussion

3.1. Chemical analysis

The sulfate contents of U and HU were 19.5% and 32.8%, respectively, which showed that HU was obtained. The FTIR spectra of U and HU are shown in Fig. 1. The signals at 1260 and 850 cm^{-1} were indicative of the presence of sulfate ester substituted at C-3.

Table 1
^{13}C NMR signal chemical shifts (ppm) of main repeating units of U and HU: [β-D-GlcpA-(1 → 4)-α-L-Rhap3s] and [α-L-Idop A-(1 → 4)-α-L-Rhap3s].

Samples	Residues[a]	Chemical shifts (ppm)							
		C-1	C-2	C-3	C-4	C-5	C-6	C-2[b]	C-3[b]
U	G	106.4	76.8	76.8	81.5	78.4	178.3		
	R	100.4	71.5	81.5	79.0	70.4	19.6		
	I	104.1	74.0	74.8	81.1	74.0	178.3		
	R′	101.1	71.5	81.5	79.0	70.4	19.6		
HU	G	106.0	76.8	76.8	81.7	78.2	178.0		
	R	99.2	70.3	80.7	79.7	70.5	19.5	74.7	
	I	103.0	72.1	72.9	80.4	72.1	178.2	76.1	78.9
	R′	100.1	70.3	80.7	79.7	70.5	19.5	74.7	

[a] G: (1 → 4)-linked β-D-glucuronic acid; R: (1 → 4)-linked α-L-rhamnose-3-sulfate (linked with β-D-glucuronic acid); I: (1 → 4)-linked α-L-iduronic acid; R′: (1 → 4)-linked α-L-rhamnose-3-sulfate (linked with α-L-iduronic acid).
[b] Signals of modified groups.

Absorbance at 1260 cm^{-1} was attributed to the asymmetric stretching of S=O. Two other important bands were assigned at 1641 and 1045 cm^{-1} corresponding respectively to the asymmetrical stretching of C=O of uronic acids and the vibration of the C–O–C bridge of glucosides. In the spectra of HU, a new band appeared at 820 cm^{-1} originating probably from sulfate at C-2 (Yu, Liu, et al., 2003). The ^{13}C NMR chemical shift of U and HU are summarized in Table 1. As shown in Table 1, after sulfation, carbons directly attaching to electronegative sulfate ester groups would shift to lower field position, while C-1 and C-4 that were indirectly attaching to sulfate ester groups would shift to higher field position. The ^{13}C NMR spectra of HU showed that after sulfation, three new peaks at 74.7, 76.1 and 78.9 ppm could be observed. They could be assigned to carbons of the sulfated groups, C-2 (R and R′), C-2 (I) and C-3 (I), respectively. In addition, signals at 70.3, 72.1 and 72.9 ppm for unsubstituted C-2 (R and R′), C-2 (I) and C-3 (I) were also observed. These results indicated that hydroxyl groups of C-2 (R, R′ and I) and C-3 (I) were partly sulfated (Qi, Zhao, et al., 2005; Yu, Zhang, et al., 2003).

3.2. Antihyperlipidemic activity in mice

All groups had body weight's gains except for the positive control group. But the changes in body weight of all experimental and control groups showed no obvious difference ($P > 0.05$). These results suggested that U and HU did not have harmful effect on weight gain.

As shown in Table 2, compared to hyperlipidemic group, the results indicated that HU-fed group (500 mg/kg) had optimal effect on LDL-C ($P < 0.01$), but a lesser impact on TG, TC and HDL-C. However, doses of 125 and 250 mg/kg had significant effects on TC, TG, LDL-C ($P < 0.01$) and HDL-C ($P < 0.05$). More importantly, the antihyperlipidemic activity of dose of 250 mg/kg was the strongest, compared to U-fed group, TG and LDL-C concentrations were significantly decreased 28.1% ($P < 0.05$) and 28.4% ($P < 0.01$), respectively. The results proved that the antihyperlipidemic activity was not concentration dependent for HU-fed mice. As compared to positive control group, HU-fed groups showed stronger antihyperlipidemic activity but the difference was not obvious ($P > 0.05$).

The sulfated polysaccharides of seaweeds, differing chemically and physicochemically from those of land plants, may have special physiological effects on human body (Yuan et al., 2005; Zhao, Zhang, Qi, Liu, & Li, 2008). Sulfated polysaccharides are associated with the surfaces of animal cells and involved in biological activities such as cell recognition, cell adhesion or regulation of receptor functions, which are of interest in medicine (Ellouali, Boisson-Vidal, Durand, & Jozefonvicz, 1993). Polysaccharides from green alga *Ulva lactuca* have also shown the antihyperlipidemic activity (Sathivel et al., 2008). The U and HU showed a high antihyperlipidemic activity in mice but the mechanism by which they regulate TC, TG, LDL-C and HDL-C blood levels in hyperlipidemia mice is not clear. There may be an important relationship between the antihyperlipidemic effect and the antioxidant activity of U and HU. In our previous study, U and HU showed strong antioxidant activity, moreover, HU (sulfate content 32.8%) had more effective antioxidant activity than U (Qi, Zhang, et al., 2005). These results suggested that increasing sulfate content of ulvan could increase the antioxidant activity. On the other hand, in this study, the results proved that HU exhibited stronger antihyperlipidemic activity than U. Numerous works have proved the priority of sulfate groups in polysaccharides for their anticoagulant activity (Mestechkina & Shcherbukhin, 2010; Wang, Li, Zheng, Normakhamatov, & Guo, 2007). All the works have proved the priority of sulfate groups in polysaccharides for their activity. Chemical modification of polysaccharides provided an opportunity to obtain new pharmacological agents with possible therapeutic uses.

According to reports, for polysaccharide, there is another type of antihyperlipidemic mechanism: bile acid sequestrant mechanism. Yu, Liu, et al. (2003) found that bile acid excretion increased significantly when mice were fed ulvan, and exhibited the antihyoerlipidemic actions. This phenomenon may indicate another

Table 2
Effects of U, HU and inositol nicotinate on serum lipid profiles in mice supplemented with a cholesterol-rich diet.

Group		Dose (mg/kg)	TC (mmol/L)	TG (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)
1	Normal control	–	2.05 ± 0.44	1.16 ± 0.27	1.2 ± 0.13	1.28 ± 0.14
2	Hyperlipidemia	–	2.80 ± 0.39$^{\Delta\Delta}$	2.00 ± 0.39$^{\Delta\Delta}$	2.36 ± 0.40$^{\Delta\Delta}$	0.97 ± 0.29$^{\Delta\Delta}$
3	U	250	2.09 ± 0.4**	1.46 ± 0.59**	1.41 ± 0.29**	1.2 ± 0.23*
4	HU	125	2.04 ± 0.51**	1.36 ± 0.69**	1.28 ± 0.41**	1.24 ± 0.24*
5		250	2.04 ± 0.39**	1.05 ± 0.48$^{**,\#}$	1.01 ± 0.18$^{**,\#\#}$	1.25 ± 0.45*
6		500	2.36 ± 0.95	1.48 ± 1.11	1.24 ± 0.53**	1.16 ± 0.34
7	Positive control	500	2.19 ± 0.53**	1.33 ± 0.44**	1.35 ± 0.18**	1.13 ± 0.13*

* $P < 0.05$: compared to hyperlipidemia control group.
** $P < 0.01$: compared to hyperlipidemia control group.
$^{\#}$ $P < 0.05$: compared to ulvan group.
$^{\#\#}$ $P < 0.01$: compared to ulvan group.
$^{\Delta\Delta}$ $P < 0.01$: compared to normal group.

mechanism by which polysaccharides can act as stimulators of bile acid synthesis. Most of the bile acids are resorbed from the small intestine and returned to the liver so that the bile acid pool remains essentially constant. Bile acid sequestering resins act in the small intestine by interrupting the enterohepatic circulation and increasing the fecal excretion of bile acids so that fewer bile acids are returned to the liver. This increases the synthesis of bile acids, and the lose of bile acids is compensated for by the oxidation of more hepatic cholesterol, the only precursor to bile acids, thereby decreasing the total blood cholesterol levels (Kul-Lee et al., 1999; Sucking, 1988).

4. Conclusion

In conclusion, U and HU possessed antihyperlipidemic activity. In addition, HU exhibited stronger antihyperlipidemic activity than U. It was likely that the sulfate content had significant effect on the antihyperlipidemic activity. However, the mechanisms of HU on antihyperlipidemic activity need to be further researched.

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