Research Article

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Antihyperglycemic and antihyperlipidemic effects of low-molecular-weight carrageenan in rats

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Abstract: This study investigated the antihyperglycemic and antihyperlipidemic effects of low-molecular-weight carrageenan (LC) on rats fed a high-fat diet. Wistar rats were divided into five groups: normal control group (NC), high-fat diet control group (HC), carrageenan-treated control group (CC), 1% LC group (1% LC), and 3% LC-groups (3% LC). Body weight, food intake, fecal weight, blood glucose, and serum lipid levels were measured. After 30 days, body weight significantly decreased in the LC-treated groups than in the HC group. Moreover, in the LC-treated groups, postprandial blood glucose, total cholesterol, triglyceride, and low-density lipoprotein cholesterol (LDL-C) levels decreased, whereas high-density lipoprotein cholesterol (HDL-C) levels increased. From this study, our data suggest that LC has antihyperglycemic and hypolipidemic effects when compared to carrageenan, likely related to its increased absorption due to its lower molecular weight.

Keywords: Carrageenan; antihyperglycemic; hypolipidemic

1 Introduction

Numerous studies have reported that obese individuals are more likely to develop diabetes, dyslipidemia, hypertension, and coronary artery disease, which are considered lifestyle-based diseases [1]. Consequently, there is increasing interest in the use of functional foods as protective agents against obesity, because they are easily available, non-toxic, inexpensive, biodegradable, and biocompatible [2]. Sulfated polysaccharides are among these functional foods, and have presented a variety of potentially therapeutic biological effects on lifestyle-based diseases [3]. Considerable research has already demonstrated the strong antioxidant, antitumor, immunostimulatory, anti-inflammatory, lipid-lowering, antiviral, antibacterial, and medical applications of sulfated polysaccharides [4].

Carrageenan is a high-molecular-weight sulfated polysaccharide (>200 kDa) that is extracted from red algae. Native carrageenan is considered to be harmless and has widespread application in the food industry as a thickening agent, gallant, and stabilizer [5]. Previous studies have shown that carrageenan can inhibit angiogenesis and metastasis, and can also act as an immune regulator, and stimulate the activity of oxidizing enzymes [6]. In addition, carrageenan has been shown to inhibit the absorption of other nutrients, with several favorable effects, including hypoglycemia and antitumor and immunomodulatory activities [7]. Furthermore, compared with carrageenan, its derivatives possess greater biological effects including antitumor, antioxidant, anticoagulant, immunoregulatory, and anti-angiogenic activities [8]. The sulfate content and molecular weight of carrageenan have been reported as the main factors that influence its antioxidant and antitumor activities [9]. However, degraded carrageenan (<50 kDa) induces inflammation and has been widely used to induce models of colitis in several species [5].

However, little is known of whether low-molecular-weight carrageenan (LC) (<2000 Da) has antihyperglycemic and hypolipidemic effect. The specific goals of this study were to determine changes in blood glucose and serum lipid levels in rats that were fed a high-fat diet together with LC in comparison to carrageenan. Additionally, we also sought to investigate the effects of dietary LC on intestinal absorptive function and the associated relevant mechanisms.

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2 Materials and Methods

2.1 Sample Preparation

LC was extracted from *Eucheuma spinosum* (Hailongda Company, Yantai, Shandong, China). *Eucheuma* was prepared by cleaning under running water, open-air drying, and then chopping into thin slices that were hydrolyzed in hydrochloric acid (pH 2.5) for 24 hours. The mixture was then filtered and hydrolyzed again in hydrochloric acid (pH 4.5) for 12 hours, then filtered and washed in distilled water. Treated *Eucheuma* was then saponified in an alkaline solution (pH 8) for 3 hours, filtered, and washed in distilled water again, then dried in a dehydrator and grounded into small particles in a disintegrator. The powder obtained was low-molecular-weight carrageenan. This method of preparation has been registered by a national patent (patent number ZL 201110419627.7) in China [10].

2.2 Molecular Weight of LC

The molecular weight (MW) was determined by high-performance gel permeation chromatography (HPGPC) at the Institute of Oceanology, Chinese Academy of Sciences, using a Perkin-Elmer series 200 instrument equipped with a refractive index detector and a gel filtration column (TSK-Gel 5000 PW 7.8 × 300 mm connected to a TSK PWH 5 × 75 mm guard column; Tosoh, Japan). Samples were eluted with 0.2 M NaCl mobile phase at a flow rate of 1 mL/min [11]. Using gel permeation chromatography in combination with light-scattering measurements conducted at the Institute of Oceanology (Chinese Academy of Sciences), the molecular weight was confirmed as 1398 Da.

2.3 Animals and Diets

Healthy Wistar rats (weight 140–160 g) were purchased from the Experimental Animal Center in Shandong Province and each rat was maintained in a metabolic cage. All animals were acclimatized to an environment with controlled temperature (22–25°C), humidity, and light/dark cycles, and allowed free access to food and water for 1 week prior to the study. All study animals were housed according to the regulations specified for the welfare of experimental animals.

Both ordinary (total calories 3.80 kcal/g, 19% fats, 55% carbohydrates, 22% proteins, 7% ash, and 5% cellulose) and high-fat (total calories 5.22 kcal/g, 3.5% cholesterol, 10% lard, 0.2% propylthiouracil, 0.5% sodium cholate, and 5% refined sugar) diets were purchased from the Experimental Animal Research Institute of the Chinese Academy of Sciences, and 1.0 g carrageenan per 100 g high-fat diet was mixed into the diet to prepare the CC diet cake; 1 g LC was mixed into 100 g high-fat diet to obtain the 1% LC diet cake; and 3 g LC was mixed per 100 g high-fat diet to obtain the 3% LC diet cake.

This study was approved (approval no. 2013100508) by the Laboratory Animal Care Committee of Qingdao Medical University, and all animal experiments were conducted in accordance with the Guidelines for Care and Use of Laboratory Animals at the Qingdao Medical University.

Ethical approval: The research related to animals use has been complied with all the relevant national regulations and institutional policies for the care and use of animals.

2.4 Study Procedure

Fifty male (5 weeks old) rats were randomly selected and divided into five groups, with 10 rats in each group. The normal control group (NC) was fed an ordinary diet, the high-fat group (HC) was fed a high-fat diet, the treated control group (CC) was fed the CC diet, and the low- and high-dose groups were fed 1% and 3% LC diets, respectively.

During the treatment period, fasting blood glucose was measured at 06:00 on days 1 and 31, and postprandial blood glucose was measured at 18:00 in the evening on days 1 and 30. A portable glucometer and blood glucose test strips were purchased from Johnson Medical Instrument Co., Ltd. (Shanghai, China). Body weight, fecal weight, and food intake of rats were measured at 8:00, 13:00, and 17:00 daily, and food was provided at 6:00, 11:00, and 15:00 daily. Food availability (i.e., degree of absorption and utilization) and fecal moisture capacity were also calculated according to the following formulas: Food availability (%) = (Final body weight − Initial body weight) / Food intake in experimental session × 100, and Fecal moisture capacity = (Fecal wet weight − fecal dry weight) / Fecal wet weight × 100. Feces were collected to determine fecal wet weight followed by drying to determine fecal dry weight. All weights were measured daily using an electronic balance.

Thirty days later, the rats were sacrificed by exarticulation after fasting for 12 hours and 7 mL of blood was collected from the abdominal aorta. Blood samples

were transferred directly to centrifuge tubes, allowed to clot at room temperature, and serum was obtained by centrifugation to determine levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C). Reagents for TG, HDL-C, and LDL-C detection were purchased from Beijing Chengxinde Biochemistry Reagent Co. (Beijing, China). Reagents for TC detection were purchased from Nanjing Jiancheng Biochemistry Reagent Co. (Nanjing, China).

2.5 Statistical Analysis

Data are expressed as mean ± standard deviation (SD). Statistical analyses were conducted with statistical SPSS v16.0 software. One-way analysis of variance (ANOVA) and Bonferroni's range test were used for quantitative data analysis. Statistical significance was set at $P<0.05$.

3 Results and Discussion

3.1 Results of bodyweight, caloric intake, TC, TG, HDL-C, and LDL-C

During the experiment, the body weight progressively increased (Figure 1). During the first 15 days of this study, the mean body weight of animals in the HC group was significantly higher than that of the NC group, and this was attributed to the effect of high-fat diet. At day 20, the body weight in the 3% LC group was significantly lower than that in the HC group, which indicated the inhibitory effect of LC on weight gain induced by high-fat diet. On Day 30, a significant difference was observed in body weight gain of animals, which gradually increased in the 3% LC, 1% LC, and CC groups.

The effects of LC in combination with a high-fat diet on caloric intake, weight gain, and food availability of rats are shown in Table 1. Rats in the HC group had a significantly increased caloric intake and weight gain compared to those in the NC group, consistent with a high-fat diet which characteristically increases the body weight and caloric intake. Rats in the CC, 1% LC, and 3% LC groups exhibited a gradual yet significant decrease in caloric intake and food availability as compared to the HC group ($P<0.05$).

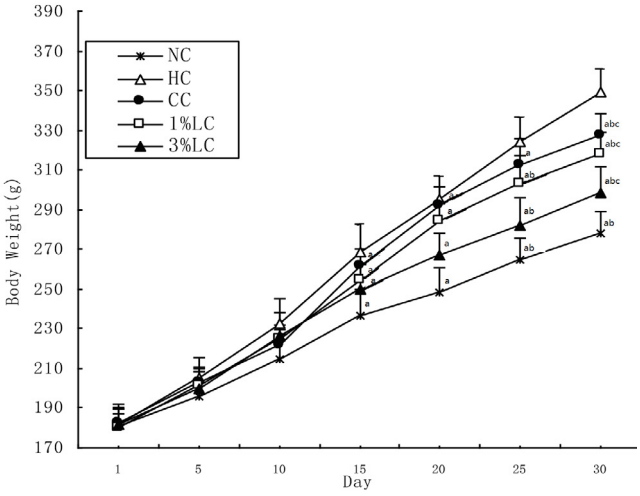

Figure 1. Effects of LC on changes of body weight in rats over 30 days. Body weight and food intake of 50 rats from five groups were measured daily. The five groups were: normal control group (NC) fed an ordinary diet; the high-fat group (HC) fed a high-fat diet; the treated control group (CC) fed 1 g carrageenan per 100 g high-fat diet; and the low- and high-dose study groups (1% LC and 3% LC) fed 1 and 3 g LC per 100 g high-fat diet, respectively. Mean± standard deviation (vertical lines). a: compared with HC, $P<0.05$; b: compared with CC, $P<0.05$; and c: compared with 1% LC, $P<0.05$.

The effects of LC combined with high-fat diet on fecal wet weight, fecal dry weight, and fecal moisture capacity in rats are shown in Table 2. The data show a significant decrease of fecal dry weight for rats in the 1% LC and 3% LC groups, compared to those without the LC combined high-fat diet ($P<0.05$). However, rats in the CC, 1%LC, and 3%LC groups had a significant gradual increase in fecal moisture capacity compared to the HC group ($P<0.05$),

Table 1. Effects of LC on the rate of food utilization in rats ($\overline{x}$ ±SD)

Group	Food intake (kcal)	Body weight gain (g)	Food availability (%)
NC	780.98±29.97[a]	97.62±8.91[a]	47.50±2.12[a]
HC	1737.84±35.16	167.72±7.96	50.38±3.07
CC	1631.82±38.12[a]	145.09±12.50[a]	46.41±2.66[a]
1% LC	1580.36±40.23[a]	137.35±10.11[ab]	45.37±2.24[a]
3% LC	1475.43±32.14[ab]	116.37±7.75[abc]	41.17±3.01[abc]

[a]compared with HC, $P<0.05$; [b]compared with CC, $P<0.05$; [c]compared with 1% LC, $P<0.05$

Table 2. Effects of LC on fecal moisture capacity in rats ($\overline{x}$ ±SD)

Group	Fecal wet weight (g/d)	Fecal dry weight (g/d)	Fecal moisture capacity (%)
NC	6.82±1.29[a]	5.14±1.49[a]	24.63±9.82[a]
HC	7.71±0.97	6.41±0.84	16.86±7.24
CC	7.47±1.53	5.48±0.75	25.30±8.49[a]
1% LC	7.42±1.67	5.32±1.01[a]	28.30±7.16[a]
3% LC	7.12±1.04	4.58±0.77[ab]	35.67±5.33[abc]

[a]compared with HC, $P<0.05$; [b]compared with CC, $P<0.05$; [c]compared with 1% LC, $P<0.05$

Table 3. Effects of LC on blood glucose in rats ($\overline{x}$ ±SD, mmol/L)

Group	Initial		Final	
	FBG	PBG	FBG	PBG
NC	4.91±0.86	6.82±1.29	4.83±1.05	6.00±0.43[a]
HC	5.02±1.05	7.11±0.97	4.97±1.31	7.04±0.68
CC	4.79±0.92	7.42±0.67	4.89±1.16	6.44±0.43[a]
1% LC	4.86±1.02	7.27±1.03	5.04±1.22	6.19±0.70[a]
3% LC	4.71±0.95	7.12±1.04	5.11±1.09	5.52±0.64[abc]

[a]compared with HC, $P<0.05$; [b]compared with CC, $P<0.05$; [c]compared with 1% LC, $P<0.05$

Table 4. Effects of LC on lipid levels in rats ($\overline{x}$ ±SD)

Group	TG (mmol/L)	TC (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)
NC	1.82±0.55[a]	1.28±0.16[a]	28.26±2.89[a]	47.03±3.43[a]
HC	3.51±0.69	1.93±0.29	47.50±2.97	28.18±3.79
CC	2.77±0.51[a]	1.61±0.22[a]	39.89±4.14[a]	30.24±5.42
1% LC	2.62±0.55[ab]	1.57±0.35[ab]	38.03±4.79[a]	32.30±5.04[a]
3% LC	2.09±0.32[ab]	1.11±0.33[ab]	34.80±3.90[ab]	34.08±4.31[a]

[a]compared with HC, $P<0.05$; [b]compared with CC, $P<0.05$; [c]compared with 1% LC, $P<0.05$

which suggest that LC in combination with a high-fat diet can lead to reduction in fecal dry weight, possibly due to decreased food intake. However, there was no significant effect in the fecal wet weight of rats fed a high-fat diet with or without LC ($P>0.05$). As shown in Table 2, the proportional reduction in fecal dry weight was much greater than that of fecal wet weight.

The effects of LC in combination with a high-fat diet on fasting blood glucose and postprandial blood glucose of rats are shown in Table 3. Initially, the levels of fasting and postprandial blood glucose in rats of the different groups were not significantly different ($P>0.05$). Toward the end of the experiment, the postprandial blood glucose levels in rats of CC, 1% LC, and 3% LC groups were significantly lower than those of the HC group (Table 3). However, the fasting blood glucose of these groups remained unchanged ($P>0.05$).

The effects of LC in combination with a high-fat diet on TC, TG, HDL-C, and LDL-C of rats are shown in Table 4. A noticeable increase was evident in TC and TG of rats fed a high-fat diet when compared to rats in the NC group ($P<0.05$), indicating that administration of a high-fat diet influences serum lipid levels. Thus, a high-fat diet significantly increased the measured serum TG, TC, and LDL-C, whereas it decreased serum HDL-C. Additionally, there was a significant decrease in the TG, TC, and LDL-C of rats in the 1% LC and 3% LC groups compared to measurements from the HC group ($P<0.05$). This reduction was significant, and appeared to be dose-dependent following increasing dietary levels of LC to 3% in the experimental groups, as compared to the HC group. There was also a significant increase in the HDL-C levels of rats in the 1% LC and 3% LC groups, when compared to the HC group ($P<0.05$).

4 Discussion

In the present research, we found that the rate of weight gain in the 1% LC and 3% LC groups was lower than those of the other groups, suggesting that LC was effective in preventing an increase in body weight and producing decreased obesity of rats fed a high-fat diet. Moreover, the effect with LC is stronger than that of carrageenan. This result corroborates the findings of Athanasios et al., who reported that decreased intestinal nutrient absorption led to reduced weight gain [12]. We thus propose that the lower rate of weight gain may be attributed to the difference in caloric intake among groups on different diets This is perhaps owing to the mechanistic involvement of fiber since it is known that soluble fiber is very viscous and can decrease caloric intake by their effect on the gastric emptying time. This finding is also in accordance with previously published data of Athanasios et al., who demonstrated that the addition of polysaccharides improved lipid metabolism and significantly lowered the nutrient availability following a high-fat diet [12].

The results of this study also showed that the use of LC in a high-fat diet increased fecal moisture capacity, and was beneficial for the elimination of feces, and inhibition of intestinal nutrient absorption. As shown in Table 2, although both carrageenan and LC can lower caloric intake and inhibit food availability, LC produced a significantly stronger effect than carrageenan. The increase of the fecal moisture content in the LC group is consistent with the hypothesis that LC increases the viscosity of intestinal contents and thereby decreases nutrient absorption and food intake. Additionally, the results also showed that the solid content of feces was reduced whereas water uptake increased.

In this study, in spite of the increase in postprandial blood glucose that was induced by high-fat diet, rats that received 1% LC or 3% LC diets showed lower postprandial blood glucose as compared to those in the high-fat diet group. These findings indicate that both LC and NC can inhibit the increase in postprandial blood glucose of rats fed with high fat diet, but had no effect on fasting blood glucose. Furthermore, 3% LC showed a greater antihyperglycemic effect than that of carrageenan. Our results also show that the antihyperglycemic effect of low molecular carrageenan is dose dependent.

Diets rich in soluble fiber result in increased viscosity of intestinal content as these fibers are molecules that bind water and possess the property of forming colloidal gels. This then decreases the association of food with the intestinal mucosa and the enzymatic digestion rate, consequently decreasing the intestinal absorption of nutrients

Our results indicated that this reduction in the TG, TC, and LDL-C of rats in the 1% and 3% LC groups was significant, and appeared to be dose-dependent after increasing dietary levels of LC to 3% in the experimental groups, as compared to the HC group. Although a high-fat diet resulted in an increase of serum lipids, supplementation of the high-fat diet with LC was very effective in preventing this increase. Serum HDL-C is known to be a beneficial factor, protective against coronary heart disease and in our study, was significantly depressed in rats that were fed a high-fat diet. However, supplementation of the high-fat diet with LC produced a significant elevation in HDL-C as compared to rats fed only a high-fat diet.

Dietary LC caused a dose-dependent reduction in body weight and food availability, and an increase in fecal moisture capacity in rats that were fed a high-fat diet. In this experiment, in spite of the observed increase in lipid parameters (TC, TG, and LDL-C) induced by a high-fat diet, rats that were fed LC together with the high-fat diet exhibited lower serum TG, TC, and LDL-C. This suggests that LC plays a role in preventing the increase of TC and TG in rats fed a high-fat diet, and this could potentially be an anti-obesity effect. These results are consistent with earlier reports of the activity of soluble polysaccharides in reducing cholesterol levels with the ingestion of viscous soluble fibers such as pectin [13]. Dietary soluble fiber is known to increase the viscosity of intestinal content through the adsorption of water and formation of colloidal gels. This reduces the interaction of food with the intestinal mucosa and the rate of enzymatic digestion, consequently decreasing intestinal nutrient absorption. Results of this study concur with the data published by other researchers, who found that guar gum not only reduces appetite by filling intestines through this mechanism, but also decreases blood lipid levels by adsorbing cholic acid [8]. The observation that LC has a greater hypolipidemic effect than carrageenan is likely related to the fiber content of LC, but also, importantly, its increased absorption resulting from its lower molecular weight.

According to our data, postprandial blood glucose levels in rats that were fed a high-fat diet combined with LC were lower than those in rats fed only a high-fat diet or a high-fat diet combined with carrageenan. We hypothesize that other bioactivities of LC may be favorable factors in decreasing postprandial blood glucose by inhibition of nutrient absorption and acceleration of peristalsis in the small intestine. The most important novel observations from this study were the hypolipidemic and antihyperglycemic effects of LC. Many papers support the

process of carrageenan induced intestinal inflammation and disruption of intestinal epithelial monolayers [14, 15]. With this in consideration, the observed weight loss and lower food intake in the LC groups may be attributed to carrageenan toxicity in the gut. However, our examination of small intestinal tissues did not reveal signs of inflammation. During the course of the experiment, the integrity of small intestinal mucosa was maintained, without significant inflammation, bleeding, or edema of the villi. Given that the dose of LC or carrageenan was low and the study duration short, detection of inflammation may not have been reasonably expected.

In summary, LC not only increases intestinal content, but also inhibits intestinal mucosal absorption, and accelerates peristalsis in the small intestine. These processes appear to be associated with reduced serum lipid levels, also reduces postprandial blood glucose and body weight. Collectively, our findings indicate a potent protective and therapeutic effect of LC against hyperlipidemia and obesity. A possible mechanism for the beneficial effect of LC on glucose and lipid levels may be related to its increased absorption owing to its lower molecular weight. These hypothetical mechanisms will need to be validated in further studies.

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