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Structural modulation of gut microbiota by chondroitin sulfate and its oligosaccharide

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ABSTRACT

Chondroitin sulfate (CS) as a dietary supplement and a symptomatic slow acting (SYSA) drug has been used for years. Recently, CS has been demonstrated to be readily degraded and fermented *in vitro* by specific human gut microbes, hinting that dietary CS may pose a potential effect on gut microbiota composition *in vivo*. However, until now, little information is available on modulations of gut microbiota by CS. In the present study, modulations of gut microbiota in Kunming mice by CS and its oligosaccharide (CSO) were investigated by high-throughput sequencing. As evidenced by Heatmap and principal component analysis (PCA), the female microbiota were more vulnerable than the male microbiota to CS and CSO treatment. Besides, it is of interest to found that CS and CSO had differing effects on the abundance of *Bacteroidales S24-7*, *Bacteroides*, *Helicobacter*, *Odoribacter*, *Prevotellaceae* and *Lactobacillus* in male mice versus female mice. Collectively, we demonstrated a sex-dependent effect on gut microbiota of CS and CSO. In addition, since gut microbiota exerts a major effect on host physiology, our study highlighted that certain beneficial effects of CS may be associated with modulations of gut microbiota, which merits further investigation.

1. Introduction

Chondroitin sulfate (CS) is a family of sulfated glycosaminoglycans (GAGs) which is composed of a repeating disaccharide unit of β-1, 4-linked NULL-glucuronic acid and β-1, 3-linked *N*-acetylgalactosamine [1]. Naturally occurring CS extracted from different sources has polysaccharide chains composed of various percentages of disaccharides motifs bearing sulfate groups in different positions (Fig. 1) [1,2]. As a representative GAG that is widespread on cell surfaces and within extracellular matrices, CS has been implicated in diverse physiological events including organogenesis, cytokinesis, morphogenesis and central nervous system development [1,3]. Moreover, CS is also actively involved in the pathological process of osteoarthritis (OA) and, notably, has been used as a symptomatic slow acting (SYSA) drug or a dietary supplement for this disease in Europe and some other countries for years [1,3]. However, despite the wide use for pharmaceutical and nutraceutical applications, it should be clear that as a high-molecular-weight GAG, CS is not well absorbed after oral administration (bioavailability only 0–13%) and the precise mechanism for its therapeutic effects also remains to be elucidated [4–6].

The human gastrointestinal tract harbors a diverse ecosystem of trillions of microbial cells which is collectively termed the gut microbiota [7–9]. Accumulating evidence has demonstrated that the gut microbiota poses a widespread impact on diverse aspects of host physiology, which provides a desired lever to improve health and to prevent or treat disease [10–12]. Recently, a strong link between metabolic OA (a new subtype of OA) and changes in the composition of gut microbiota has been established [13,14]. Although failed to find causality, Collins and colleagues demonstrated a significant negative and positive relationship between the abundance of *Lactobacillus* spp. and Methanobrevibacter spp. with the development of metabolic OA, respectively [14]. Given that CS can be readily metabolized by certain gut bacteria to benefit its growth after oral dosing, this study opens a potential new way to understand the mechanism by which CS exert its anti-OA effects [15–17]. However, unfortunately, significant questions remain unanswered to address including what effect of CS has on

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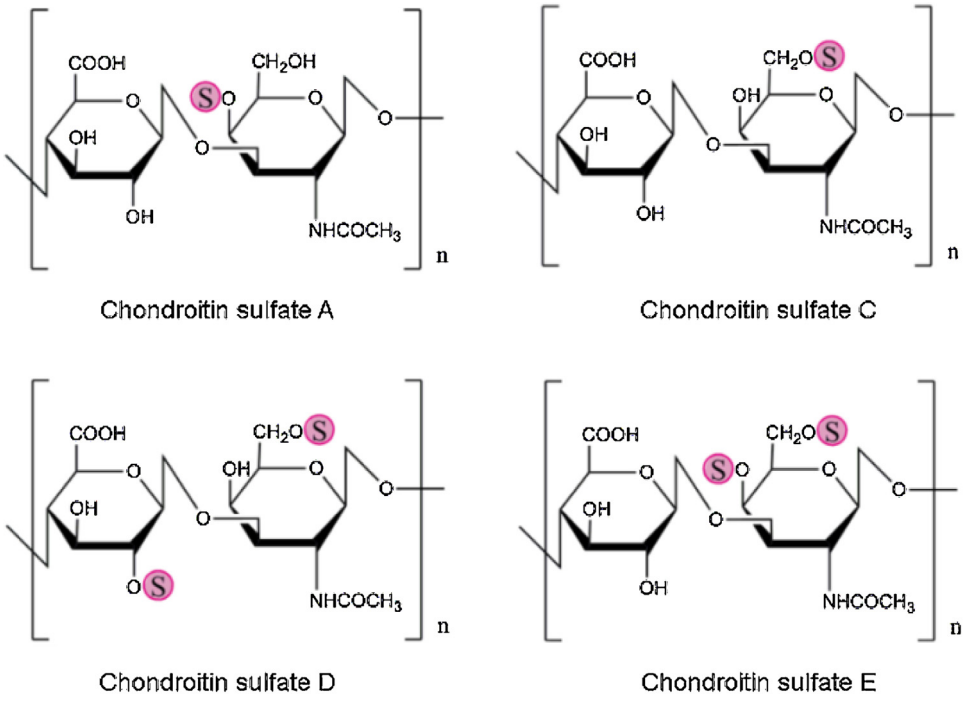

Fig. 1. Typical structures of CS. Different members from CS family are esterified by sulfate at various positions as indicated by "S" enclosed by a circle.

the overall composition of gut microbiota. Additionally, coinciding with the global prevalence of OA [18,19], the robust increase in usage of CS also warrants a more comprehensive study of its safety on modulations of the gut microbiota, since this forgotten organ plays a critical role in modulating nutrition, metabolism and immunity of its host [7,8].

In the present study, the dearth of corresponding research in previous reports, coupled with the fact that we have isolated and sequenced different CS-degrading bacteria from human feces [15], tempts us to focus on characterizing the modulations of gut microbiota by dietary CS treatment. Using high throughput sequencing analysis, we demonstrated in detail the modulations of gut microbiota by CSA, CSC and CS oligosaccharide (CSO) in both male and female mice. Our results can have important implications for understandings of the metabolism and adverse effects of CS and can potentially shed light on the anti-OA mechanism of CS.

2. Materials and methods

2.1. Materials

CSA (Mw = 24 kDa, sulfate content = 17.22%) and CSC (Mw = 130 kDa, sulfate content = 17.71%) were used as previously described [15]. CSO, constituted of ΔDi-4S, were prepared and purified as previously described [20]. All other chemicals at analytical grade were purchased from Sigma (Shanghai, China) unless otherwise stated.

2.2. Animal intervention and samples

The experimental protocol was approved by the Ethical Committee of Ocean University of China in accordance with the National Guidelines for Experimental Animal Welfare (China, 2006). A total of 48 Kunming mice (6-week old, 24 male and 24 female) were obtained from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). During the experimental session, all mice were housed with care under controlled temperature (22 ± 3 °C) and a 12 h light/dark cycle. To minimize the variation of environmental factors, all mice were feed the same batch of standard laboratory diet (Slacom, Shanghai, China) and had free access to autoclaved water. After two-week adaptation, the mice were randomly assigned to 8 groups with 6 each: Male control group (CSNM), male CSA group (CSAM), male CSC group (CSCM), male CSO group (CSOM), female control group (CSNF), female CSA group (CSAF), female CSC group (CSCF) and female CSO group (CSOF). In the following 6 weeks of treatment, the CSNM and CSNF groups were given an oral administration of normal saline once a day while the other groups were given different isoforms of CS with a dosage of 150 mg/kg by gavage.

At the end of the animal experiment, all mice were anaesthetized and sacrificed by cercal dislocation after an overnight fasting. The cecal contents were collected and stored at −80 °C before being analyzed.

2.3. Amplification and high throughput sequencing of gut bacteria

Total DNA from each cecal sample of mice in all 8 groups was extracted using QIAamp DNA Stool Mini Kit (Qiagen, Hamburg, Germany) according the manufacturer's instructions. Deproteinization by proteinase K treatment and DNA purification with QIAamp Mini Spin columns were respectively performed following the kit protocol. The final DNA concentration was determined by using a NanoDrop ND-2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA). According to different treatment protocols, all extracted DNA samples were pooled at equal concentrations to generate 8 samples in total before being analyzed. The V3-V4 hypervariable regions of the 16S rRNA gene from 8 groups were amplified with universal primers: 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). PCR reaction mixture was consisted of 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 2 μL of 10 × FastPfu Buffer, 0.2 μL of FastPfu Polymerase and 10 ng of template DNA. The PCR reactions were conducted in a thermocycler PCR system (GeneAmp 9700, ABI, USA) using the following program: 3 min of denaturation at 95 °C, 27 cycles of