三重大学

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Mie University

NEWS RELEASE

Rhamnan sulphate from green algae *Monostroma nitidum* improves constipation with gut microbiome alteration

More effective on obese people

- **Rhamnan sulphate*[1] increases defecation volume and calorie consumption by defecation in obese mice**
- **Placebo-controlled, double-blind, comparative human clinical study*[2] showed improved constipation in subjects taking rhamnan sulphate**
- **The constipation-improving effect of rhamnan sulphate is more pronounced when the BMI (index of obesity) is above average.**
- **Subjects who ingested rhamnan sulphate showed a tendency toward improvement in intestinal flora, including a decrease in bad bacteria.**
- **Based on the results of the research, we plan to apply rhamnan sulphate extracted from human wheatgrass to the development of functional foods and supplements.**

Monostroma nitidum

Abstract

A research group led by Yasuhito Shimada, M.D., Ph.D., an associate professor at the Mie University Graduate School of Medicine, has discovered that rhamnan sulphate contained in the green alga *Monostroma nitidum* (Hitogusa in Japanese) is effective in improving constipation in humans.

Monostroma nitidum is one of the specialties of Mie Prefecture in Japan, which boasts 60-70% of the domestic production.

Rhamnan sulphate, a water-soluble dietary fiber composed mainly of rhamnose found in *Monostroma nitidum*, is known to have anti-clotting, anti-viral, and anti-inflammatory effects on vascular endothelial cells. In 2016, our research group also discovered that it has anti-obesity effects in a study on zebrafish (Zang L, Shimada Y, Tanaka T, Nishimura Y. Rhamnan sulphate from Monostroma nitidum attenuates hepatic steatosis by suppressing lipogenesis in a diet-induced obesity zebrafish model. *J Funct Foods*. 2015;17:364-370.).

In this study, Mie University and Konan Chemical Manufacturing Co., Ltd. (Headquarters: Yokkaichi City, Mie Prefecture) jointly conducted mouse and human clinical trials using rhamnan sulphate, and found that rhamnan sulphate improves

intestinal microflora (intestinal flora), resulting in improved constipation. In addition, metagenomic analysis*3 and network analysis of the intestinal microflora using a next-generation sequencer revealed that rhamnan sulphate may reduce inflammation in the intestines and promote the enhancement of biological defense mechanisms.
The results of this study were published in the online edition of Scientific Reports on July 5, 2021.

Background

According to the 2016 National Survey on Basic Living Conditions, the rate of people with subjective symptoms of constipation (complainants) in Japan is reported to be 2-5%. In particular, it can be seen that more and more people suffer from constipation due to disordered dietary habits caused by unbalanced nutritional intake associated with recent food insatiability, lack of exercise, dieting, and constant stress. The state of the intestinal microflora plays a major role in the health of the stomach, including constipation, and great expectations are placed on bifidobacteria, lactic acid bacteria, and other bacteria to improve the environment.

As mentioned above, it has been known that rhamnan sulphate has a variety of effects, but it has not been evaluated in mammalian animals or humans. In the present study, we first examined whether the anti-obesity effect of rhamnan sulphate could also occur in mice, and found an increase in excreted calories (fecal volume), which led us to recognize the possibility of its effect on constipation, and to conduct human trials.

Achievements

Rhamnan sulphate suppressed weight gain in obese mice with reduction of bllod triglycerides and total cholesterol. In addition, the amount of feces and calories excreted increased significantly, suggesting that rhamnan sulphate intake affects the intestinal microflora.
Next, in a human clinical study, a placebo-controlled, randomized, double-blind, parallel-group comparison study was conducted on constipated subjects, and the following five points were clarified.

① By taking rhamnan sulphate (100 mg/day) for two weeks, the frequency of defecation increased significantly compared to placebo, i.e., constipation improvement was observed.

② Furthermore, sub-cluster analysis by BMI (above/below average) showed that both the number of bowel movements and the number of days of defecation increased more in subjects with higher BMI (fatter than average) than in placebo. In other words, rhamnan sulphate was more effective for obese people.

③ Metagenomics analysis of the intestinal microflora in the feces using a next-generation sequencer showed that Firmicutes tended to decrease, while Bacteroides tended to increase. It is known that the more obese a person is, the more Firmicutes is present, and the less obese a person is, the more Bacteroides is

present, indicating that the intestinal microflora has been improved. In addition, clostridia (Firmicutes), which produce medium-chain fatty acids that increase the absorption of water in the intestine (one of the causes of constipation), were reduced by the intake of rhamnan sulphate.

④ It is known that the number of bacterial species in the intestines increases with constipation. When the number of types of bacteria in the intestines of the subjects was stratified by "above average/below average," it was found that subjects with more types of bacteria in their intestines had a stronger effect on constipation.

⑤ By network analysis combining PICRUSt and KEGG pathways, the functions of the intestinal microbiota altered by rhamnan sulphate were inferred. The results showed that rhamnan sulphate intake activated the "cytochrome p450-mediated excretion of foreign substances" pathway, "biological defense against invasive bacteria," and the "biomolecular NAD synthesis" pathway, which has already been reported to have therapeutic effects on constipation, suggesting a possible mechanism for the ameliorating effects of rhamnan sulphate.

Future plans

Our human clinical study has demonstrated that the effects reported in cultured cells and zebrafish studies are actually effective in humans. Within the next year, we aim to develop the first functional food product that utilizes the effects of rhamnan sulphate extracted from *Monostroma nitidum*. Furthermore, since the therapeutic effect of rhamnan sulphate was found to be related to the degree of obesity and the diversity of bacterial flora, we plan to develop supplements tailored to each person's condition.

We plan to continue research on the effects of rhamnan sulphate that have not yet been proven in mammals and humans, such as immune activation and suppression of vascular inflammation, to further demonstrate the therapeutic effects of rhamnan sulphate in humans.

Terminology

*[1] Rhamnan sulphate
It is a kind of sulphated polysaccharides with rhamnose as its backbone found in green algae. And among green algae, the content of rhamnose is the highest in *Monostroma nitidum*. It is known to have a variety of biological regulatory effects, including antiviral, anticoagulant, antihyperglycemic, cholesterol-lowering, and anti-obesity effects.

*[2] Double-blind comparative study
A type of trial design in which all parties involved in the trial (the person administering the drug and the person taking the drug) are completely unaware of the type of drug being administered. It is the most common method of comparative testing to verify the therapeutic efficacy of a new drug (test drug) and is also known as a double blind test.

*[3] Metagenomics analysis
A method to comprehensively analyze genomic DNA extracted from clinical, fecal, and environmental samples to elucidate the species composition and functions

of bacterial flora.

*4 Network analysis

It is an analytical method to identify which pathways are activated (or repressed) by fitting comprehensive expression data of metabolites and gene products to a known network. In this study, we used PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) and Kyoto University KEGG (Gene Product and Metabolite Pathway Map Database).

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