DOI: 10.1002/pul2.12266

RESEARCH LETTER

Pulmonary Circulation Open Access | PVRI Pulmonary Vascular Research Institute | WILEY

Partially hydrolyzed guar gum suppresses the progression of pulmonary arterial hypertension in a SU5416/hypoxia rat model

Takayuki J. Sanada[1,2] | **Koji Hosomi**[1] | **Jonguk Park**[3] | **Akira Naito**[2] | **Seiichiro Sakao**[2] | **Nobuhiro Tanabe**[2,4] | **Jun Kunisawa**[1,3,5,6,7,8,9,10,11] | **Koichiro Tatsumi**[2] | **Takuji Suzuki**[2]

[1]Laboratory of Vaccine Materials and Laboratory of Gut Environmental System, Microbial Research Center for Health and Medicine, National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), National Institutes of Biomedical Innovation, Health, and Nutrition (NIBIOHN), Osaka, Japan

[2]Department of Respirology, Graduate School of Medicine, Chiba University, Chiba, Japan

[3]Artificial Intelligence Center for Health and Biomedical Research, National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), Osaka, Japan

[4]Department of Respirology, Chibaken Saiseikai Narashino Hospital, Narashino, Japan

[5]Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan

[6]Graduate School of Medicine, Osaka University, Osaka, Japan

[7]Graduate School of Dentistry, Osaka University, Osaka, Japan

[8]Graduate School of Science, Osaka University, Osaka, Japan

[9]Department of Microbiology and Immunology, Graduate School of Medicine, Kobe University, Hyogo, Japan

[10]International Vaccine Design Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan

[11]Research Organization for Nano and Life Innovation, Waseda University, Tokyo, Japan

Correspondence
Takayuki J. Sanada, Laboratory of Vaccine Materials and Laboratory of Gut Environmental System, Microbial Research Center for Health and Medicine, National Institutes of Biomedical Innovation, Health, and Nutrition (NIBIOHN), 7-6-8 Saito-Asagi, Ibaraki, Osaka 567-0085, Japan.
Email: jujo-sanada@nibiohn.go.jp

Funding information
Ministry of Health and Welfare of Japan, and public/private R&D investment strategic expansion program PRISM, Grant/Award Number: 20AC5004; The Japan Agency for Medical Research and Development (AMED), Grant/Award Number: JP20gm1010006h0004; A Grant-in-Aid for

Abstract

This study investigated the effects of partially hydrolyzed guar gum (PHGG) on the development of pulmonary arterial hypertension using a SU5416/hypoxia rat model. Our results demonstrated that PHGG treatment suppressed the development of pulmonary hypertension and vascular remodeling with an altered gut microbiota composition.

KEYWORDS
Akkermansia, bacteroidetes S27-7, dietary fiber, gut dysbiosis, prebiotics

Scientific Research C from the Japan Society for the Promotion of Science (JSPS), Grant/Award Numbers: 17K09604, 20K08534

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a life-threatening disease characterized by elevated pulmonary arterial pressure and resistance, which induces right heart failure and death.[1] Pulmonary arterial remodeling, characterized by intimal cell proliferation and medial thickening of the pulmonary arteries (PAs), is a pathological feature of PAH. Subsequent PA obstruction due to remodeling is closely associated with elevated pulmonary arterial pressure.[2] Remodeling is considered to be induced by an abnormal inflammatory response[1]; however, the origin of the inflammation and its mechanisms remain unclear.

The gut microbiota is an ecological community of symbiotic microorganisms that play an important role in producing vitamins and maintaining gut barrier functions and the immune system.[3] Abnormal alterations, known as gut dysbiosis, are related to the development of cardiovascular diseases.[3] Recently, gut dysbiosis has been observed in PAH animal models[4] and patients with PAH[5] and chronic thromboembolic pulmonary hypertension.[6] Gut dysbiosis might impair gut barrier function and promote the influx of toxic agents derived from gut microorganisms into the systemic circulation, such as endotoxin and trimethylamine N-oxide, which might induce systemic inflammation and the development of pulmonary vascular remodeling in PAH.[5,6]

Partially hydrolyzed guar gum (PHGG) is a watery dietary fiber extracted from the albumen of *Cyamopsis tetragonolobus*.[7,8] PHGG is effective for constipation and diarrhea and has been available in the market for several decades.[7] PHGG increases the production of short-chain fatty acids by gut bacteria and protects intestinal epithelial cells from damage.[9] We hypothesized that improving gut dysbiosis using PHGG could reduce the development of PAH.

This study aimed to evaluate the effects of PHGG on PAH and vascular remodeling in an animal model with PAH.

METHODS

Details of the methods are described in Supporting Information Methods, and the study design is illustrated in Figure S1. After 1 week of habituation, all rats were randomly divided into three groups: control, SU5416/hypoxia (Su/Hx), and Su/Hx rats treated with PHGG (Su/Hx+G) (Figure S1). The PHGG powder was diluted to a concentration of 3%, and the solution was supplied to the Su/Hx+G rats as drinking water for 4 weeks. The control and Su/Hx groups received sterilized drinking water without PHGG for all the periods. At 1 week, Su/Hx and Su/Hx+G rats received a subcutaneous injection of SU5416 (20 mg/kg) and were exposed to hypoxic conditions (10% O_2) for 3 weeks. After 4 weeks, fecal samples were collected from all animals, frozen for 30 min, and stored until DNA isolation. Right heart catheterization was performed to measure the right ventricular systolic pressure (RVSP). The weight ratio of the right ventricle to the left ventricle and septum (RV/LV+S), an indicator of right heart hypertrophy,[10] was calculated. The extent of pulmonary vascular remodeling was pathologically quantified according to previous reports.[11]

DNA was isolated from the collected fecal samples, and 16S ribosomal ribonucleic acid sequencing and analysis were performed according to our previous report.[6] The details are described in Supporting Information Methods.

This study was approved by the Chiba University Instrumental Animal and Use Committee (approval numbers 4-355 and 4-379). This study was performed according to the guidelines of the Animal Research Committee of the Laboratory Animal Center, Graduate School of Medicine, Chiba University, Japan.

RESULTS

RVSP and RV/LV+S were significantly higher in Su/Hx rats than in control rats (Figures 1a,b). The percentage of obstructive vascular lesions was significantly higher in the Su/Hx rats than in the control rats (Figure 1c), which was consistent with the hemodynamic changes between the two groups. PHGG decreased RVSP and RV/LV+S values in Su/Hx+G rats compared to those in the Su/Hx group (Figures 1a,b). This was supported by the fact that the percentage of obstructive vascular lesions was significantly lower in the Su/Hx+G group than in the Su/Hx group (Figure 1c). The pathological evaluation also showed atrophy of the epithelial layers in the colon of Su/Hx rats, which was suppressed by PHGG treatment in Su/Hx+G rats (Figure 1d–f). 16S

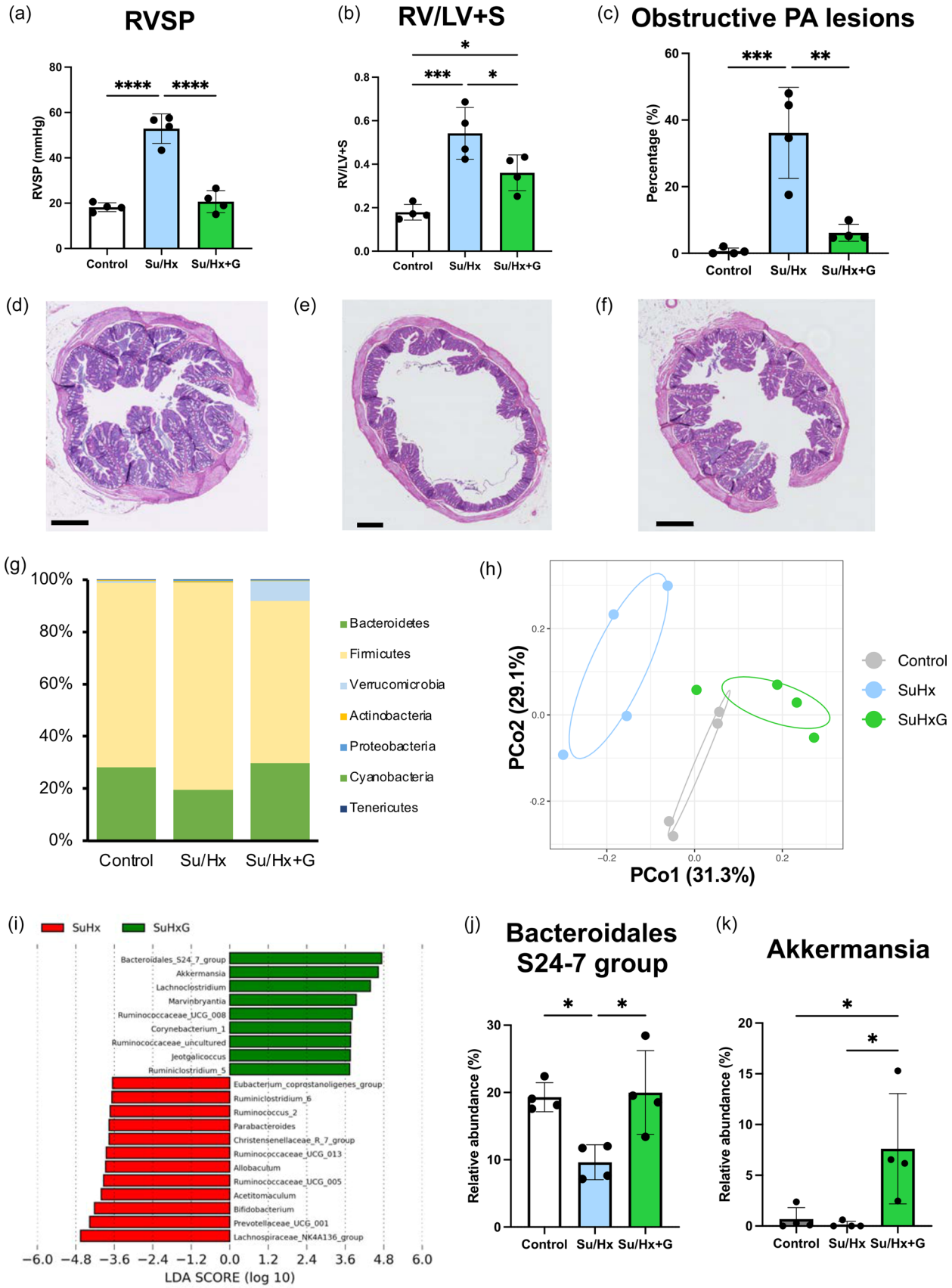

FIGURE 1 (See caption on next page).

rRNA sequencing demonstrated different gut microbiota compositions among the control, Su/Hx, and Su/Hx+G groups (Figure 1g). The principal coordinate analysis also supported the different compositions of the gut microbiota among the three groups (Figure 1h), although the α-diversities did not differ among the three groups (Figure S2). To characterize the differences in gut microbiota between the Su/Hx and Su/Hx+G groups, linear discriminant analysis effect size was performed, revealing nine bacteria characterized in Su/Hx+G rats and 12 bacteria characterized in Su/Hx rats (Figure 1i). Out of the nine bacteria, the relative abundances of the *Bacteroidales* S24-7 group and *Akkermansia* in the Su/Hx+G group were significantly increased compared with the Su/Hx group (Figures 1j,k, and S3). However, the relative abundances of Prevotellaceae UCG-001, *Acetinobmaculum*, Ruminococcaceae UCG-005, Christensenellaceae R-7 group, and *Ruminococcus 2* in the Su/Hx+G group were significantly decreased compared to those in the Su/Hx group (Figure S4).

DISCUSSION

In this study, we found that PHGG administration prevented the progression of pulmonary vascular remodeling and elevation of RVSP in Su/Hx rats, which was associated with altered gut microbiota composition.

PHGG altered the composition of the gut microbiota and restored the atrophied epithelial layer in the colon of Su/Hx rats. It is known that PHGG can improve damage to epithelial and mucin layers and suppress the inflammatory response.[12,13] Sakakida et al.[12] reported that PHGG can increase the relative abundance of *Bacteroidales* S24-7 and *Akkermansia* in rats, restore gut barrier function, and thicken the mucin layer, which is consistent with our results. *Akkermansia* and *Bacteroidales* S24-7 metabolize mucin and produce short-chain fatty acids, including acetate, propionate, and succinate.[14–16] *Akkermansia* also activates toll-like receptor 2 in intestinal epithelial cells, improving tight junctions and the gut barrier.[14] Thus, it is possible that an increase in these bacteria is associated with the suppressive effects of PHGG on PAH.

PHGG administration prevented PAH development in Su/Hx rats. Our previous study demonstrated that modifying the gut microbiota using antibiotics suppressed the development of pulmonary hypertension in Su/Hx rats,[17] indicating that gut dysbiosis can play a causal role in PAH development, and restoring the gut microbiota may be a new therapeutic option for PAH.[3] However, long-term antibiotic treatment can harm patients and does not appear to be a prebiotic agent applicable in clinical situations. A phase I clinical study on fecal microbiota transplantation in patients with PAH is ongoing,[18] although the safety and feasibility of this approach in this specific population remain unknown until the trial is completed. PHGG is commonly used in clinical settings, and its safety has already been proven. Therefore, PHGG may be an option for preventing PAH progression in the future.

The detailed mechanisms underlying the alteration of bacterial composition by PHGG remain unclear from our results. PHGG is a galactomannan with two molecules of linearly linked D-mannose and one molecule of D-galactose side chains and needs to be degenerated by β-galactomannase before general gut bacteria use.[7] The degeneration mechanism of PHGG in vivo is still unclear; however, an in vitro study showed that several bacteria with β-galactomannase, such as *Ruminococcus*, *Eubacterium*, and *Bifidobacterium*, can ferment PHGG.[19] It has been acknowledged that the genomes of *Akkermansia* and Bacteroidetes S24-7 do not code β-galactomannase,[16,19] and it seems that *Akkermansia* and Bacteroidetes S24-7 do not directly utilize PHGG. The detailed mechanism would need to be investigated in the future.

This study had some limitations. First, it is unclear whether the results are applicable to human patients with PAH as the composition of the gut microbiota in rats is quite distinct compared to human gut microbiota.[20] In this study, the effect of PHGG on PAH was examined only in a PAH animal model but not in human patients. Thus, future clinical studies

FIGURE 1 Effects of partially hydrolyzed guar gum on pulmonary arterial hypertension in SU5416/hypoxia rats. (a) Right ventricular systolic pressure (RVSP). (b) Ratio of the weight of the right to left ventricle and septum (RV/LV+S). (c) The percentage of obstructive pulmonary arteries in the lung fields. (d)–(f) Representative slices of colon stained with hematoxylin-eosin staining: (d) Control; (e) Su/Hx; and (f) Su/Hx+G groups. (g) The average of the relative abundance of gut microbiota phyla. (h) Principal coordinate analysis (multivariate analysis of variance, $p < 0.005$). (i) Linear discriminant analysis effect size in the Su/Hx and Su/Hx+G groups. The threshold for the LDA score was 3.5. The relative abundances of bacteria among control, Su/Hx, and Su/Hx+G groups: Bacteroidales S24-7 group (j) and *Akkermansia* (k). PHGG: partially hydrolyzed guar gum; Su/Hx: SU5416/hypoxia; Su/Hx+G: Su/Hx rats treated with PHGG. LDA, linear discriminant analysis.

investigating the effects on human patients with PAH are required. Second, the effects of PHGG demonstrated in this study were preventive but not therapeutic. Weeks 0–3 in the Su/Hx model correspond to the phase of disease progression from a normal state to pulmonary hypertension.[11] To evaluate the therapeutic effects of PHGG on PAH, further experiments involving the administration of PHGG to Su/Hx rats after the initial 3-week period are needed.

In conclusion, PHGG treatment suppressed PAH progression in the Su/Hx animal model. Modifying the gut microbiota with PHGG may be associated with suppressing pulmonary arterial remodeling and PAH. Thus, PHGG may be a potential prebiotic agent for suppressing the development of PAH.

AUTHOR CONTRIBUTIONS

Takayuki Jujo Sanada conceived and designed the study. Takayuki Jujo Sanada and Akira Naito performed the animal experiments. Takayuki Jujo Sanada, Koji Hosomi, Jonguk Park, and Akira Naito analyzed and visualized the data. Seiichiro Sakao, Nobuhiro Tanabe, Jun Kunisawa, Koichiro Tatsumi, and Takuji Suzuki interpreted the data. Takayuki Jujo Sanada wrote the draft. Koji Hosomi, Jonguk Park, Akira Naito, Seiichiro Sakao, Nobuhiro Tanabe, Jun Kunisawa, Koichiro Tatsumi, and Takuji Suzuki reviewed the draft.

ACKNOWLEDGMENTS

We thank Nestlé, Japan, for providing information on the characteristics of PHGG. We also thank Editage (https://www.editage.com) for English language editing. This study was supported by a Grant-in-Aid for Scientific Research C from the Japan Society for the Promotion of Science (JSPS) (Nos. 17K09604 and 20K08534), the Japan Agency for Medical Research and Development (AMED) (No. JP20gm1010006h0004), Ministry of Health and Welfare of Japan, and public/private R&D investment strategic expansion program PRISM (No. 20AC5004). The funders had no role in the study design, data collection, and analysis, decision to publish, or manuscript preparation.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ETHICS STATEMENTS

This study was approved by the Chiba University Instrumental Animal and Use Committee (approval numbers 4-355 and 4-379). This study was performed according to the guidelines of the Animal Research Committee of the Laboratory Animal Center, Graduate School of Medicine, Chiba University, Japan.

ORCID

Takayuki J. Sanada https://orcid.org/0000-0001-5725-1810

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Sanada TJ, Hosomi K, Park J, Naito A, Sakao S, Tanabe N, Kunisawa J, Tatsumi K, Suzuki T. Partially hydrolyzed guar gum suppresses the progression of pulmonary arterial hypertension in a SU5416/hypoxia rat model. Pulm Circ. 2023;13:e12266. https://doi.org/10.1002/pul2.12266