Protocol Article

Effect of probiotics, *Bifidobacterium bifidum* G9-1, on gastrointestinal symptoms in patients with type 2 diabetes mellitus: study protocol for open-label, single-arm, exploratory research trial (Big STAR study)

Yoshitaka Hashimoto, Hanako Nakajima, Shinnosuke Hata, Tomoki Miyoshi, Yukako Hosomi, Saori Majima, Naoko Nakanishi, Takafumi Senmaru, Takafumi Osaka, Hiroshi Okada, Emi Ushigome, Masahide Hamaguchi, Mai Asano, Masahiro Yamazaki, and Michiaki Fukui*

Department of Endocrinology and Metabolism, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602-8566, Japan

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Metformin is associated with risks of gastrointestinal complications in patients with type 2 diabetes. In contrast, probiotic *Bifidobacterium bifidum* G9-1 (BBG9-1) could improve the symptoms of diarrhea caused by metformin in animal models. Thus, the primary outcome of this study will be the effect of the probiotic BBG9-1 on gastrointestinal symptoms, including diarrhea, in patients with type 2 diabetes who use metformin. This open-label, single-arm, and exploratory study will examine 40 patients with type 2 diabetes who use metformin and have symptoms of constipation or diarrhea. After the baseline examination (objective 1), patients will be administered probiotic BBG9-1 for 10 ± 2 weeks. Then, examinations will be performed (objective 2). The primary outcome will be changes in the symptoms of constipation or diarrhea from objective 1 to objective 2. Secondary outcomes will include changes in gut microbiota, and correlations between changes in fecal properties and biomarkers, including HbA1c level and body mass index. This is the first study to investigate the effect of probiotic BBG9-1 on the change in the symptom of constipation or diarrhea in patients with type 2 diabetes who use metformin.

***Key Words*:** **type 2 diabetes, biguanides, probiotics, gut microbiota, gastrointestinal complications, constipation, diarrhea**

The number of patients with type 2 diabetes (T2D) is increasing worldwide.[1] Micro- and macrovascular complications are well-known complications of this disease.[1] Patients with T2D are also at an increased risk of complications from various gastrointestinal diseases, such as reflux esophagitis, constipation, and diarrhea.[2,3] Moreover, 75% of patients with diabetes have gastrointestinal complications, and 10–60% of these have constipation and 20% have diarrhea.[3,4] In addition, the use of metformin reportedly increases the risk of gastrointestinal complications.[5] Thus, many patients discontinue metformin although it exerts not only a hypoglycemic effect but also protective effects against cardiovascular events and cancer.[6]

Recently, the relationship between gut microbiota and T2D has become clear.[7–10] Dysbiosis results in chronic inflammation and insulin resistance through transfer of intestinal bacteria into the blood and reduction in short-chain fatty acids and branched-chain amino acids.[9,10] Many diet and supplements have been marketed to improve dysbiosis.[11,12] However, there is little evidence on the effectiveness of these diets and supplements. In contrast, probiotic bifidobacteria improves the balance of intestinal flora, which, in turn, improves defecation. In fact, probiotic bifidobacteria improved the symptoms of constipation and diarrhea in individuals without diabetes.[13–15] Additionally, a recent study found that probiotic *Bifidobacterium bifidum* G9-1 (BBG9-1) could improve the symptoms of diarrhea caused by metformin in an animal model.[16] However, no previous studies have revealed the effects of probiotic bifidobacteria on the symptoms of constipation and diarrhea caused by metformin in patients with T2D. Therefore, this open-label, single-arm, and exploratory research study will investigate the effects of the probiotic BBG9-1 on the symptoms of constipation or diarrhea caused by metformin in patients with T2D.

Objectives

The primary outcome of this open-label single-arm exploratory study will be the effect of probiotic *Bifidobacterium bifidum* G9-1 (Biofermin tablets®, Biofermin Pharmaceutical Co., Ltd.) on the changes in the symptoms of constipation or diarrhea. Moreover, we will investigate the effects of probiotic BBG9-1 on blood glucose control, total bile acid, fecal condition, and microbiota composition. Moreover, we will also investigate the association between microbiota composition and these markers.

Materials and Methods

Study setting. This open-label single-arm exploratory study [Effect of probiotics, **Bi**fidobacteria, on **G**astrointestinal **S**ymptoms in Patients with **T**ype 2 diabetes mellitus; open-label, single-**A**rm, exploratory **R**esearch (**Big STAR** study)] will be performed for 3 months with Japanese outpatients with T2D at the Hospital of the Kyoto Prefectural University of Medicine (Kyoto, Japan). A total of 40 patients with T2D, who use metformin and have symptoms of constipation or diarrhea, will be included. This study was regis-

*To whom correspondence should be addressed.
E-mail: michiaki@koto.kpu-m.ac.jp

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tered with the Japan Registry of Clinical Trials (jRCTs051190109) and has been approved by the Ethics Committees of the Kyoto Prefectural University of Medicine (CRB5180001). This study is to be conducted according to the Declaration of Helsinki.

Eligibility criteria. Patients must satisfy all of the following inclusion criteria and not meet any exclusion criteria. The inclusion criteria for this trial are as follows: (1) symptoms of constipation or diarrhea; (2) Gastrointestinal Symptom Rating Scale (GSRS) subdomain score (diarrhea or constipation) of three or higher; (3) diagnosis of T2D without diabetic polyneuropathy; (4) use of metformin and less than four antidiabetic agents; (5) non-use new antibiotics within 12 weeks prior to consent; (6) no history of treatment with new diet therapy interventions within 12 weeks prior to consent; (7) no changes in concomitant drugs (addition or withdrawal of concomitant drugs or change in use or dose of concomitant drugs) within 12 weeks prior to consent; (8) age of ≥20 years and <75 years upon provision of consent; and (9) written informed consent. The exclusion criteria for the trial were as follows: (1) mean weekly defecation frequency <1 or >42 times within the month prior to consent; (2) structural diseases diagnosed by colonoscopy within 5 years prior to consent; (3) history or combination of celiac and inflammatory bowel diseases; (4) HbA1c level ≥9% at the time of consent; (5) myocardial infarction, cerebral infarction, or stroke within 12 weeks prior to consent; (6) severe hepatic dysfunction (aspartate transaminase or alanine aminotransferase level ≥5 times higher than the upper normal limit); (7) severe renal dysfunction (estimated glomerular filtration rate <30 ml/min/1.73 m^2); (8) active malignant neoplasm; (9) history of bifidobacterial allergy; (10) use of any other drugs or supplements that affect intestinal function; (11) use of glucagon-like peptide-1 (GLP-1) receptor antagonists or drugs that have a high likelihood of causing gastrointestinal symptoms (prokinetic agents, gastrointestinal dysfunction therapeutic agents, antiemetic agents, or anticholinergic agents, etc.); (12) routine consumption of foods, supplements, or pharmaceutical agents including bifidobacteria (e.g., yogurt or chocolates); and (13) other conditions that the investigator or researcher thinks inappropriate for the study.

Interventions. Enrollment and follow-up visits are outlined in Fig. 1. Briefly, (1) written informed consent and provisional registration will be obtained. Participants will be selected on the basis of the aforementioned inclusion and exclusion criteria. (2) Objective 0: pre-observation. Six weeks after the provisional registration, pre-observation examinations will be performed, following which registration will begin. (3) Objective 1: before treatment observation. Further, within 8 weeks after the provisional registration, baseline examinations will be performed. After the baseline examinations, BBG9-1 administration will be started. (4) Objective 2 (10 weeks ± 2 weeks after using the drug): after treatment observation. The examinations, which are identical to those conducted at baseline, will be performed. Then, the BBG9-1 administration period will conclude. (5) Objective 3 (12 weeks ± 2 weeks after using the drug): after treatment observation. The participants will respond to the GSRS and Bristol Stool Scale and return their completed surveys to the data center. During the time from objective 1 to objective 3, all participants will be asked to complete the Bristol Stool Scale and feces questionnaire condition daily. Completed questionnaires and scales will be checked to determine administration compliance on a daily basis.

Criteria for discontinuing or modifying allocated interventions. Criteria for discontinuation of observation are follow: (1) Worse glycemic control (HbA1c ≥10%). (2) Withdraws his or her consent. (3) A serious non-conformity is found after registration. (4) Discontinue the drug because of development of complications. (5) Discontinue the drug because of illness or adverse events. (6) Pregnancy. (7) Significant nonadherence (less than 75% of all scheduled doses or more than 120%). (8) Deviation from the research protocol. (9) Physicians determine that it is appropriate to stop the study with other reason. Even if there is a deviation from the research protocol, observations should be continued to the extent possible. The data shall be handled in a blinded manner and shall be decided by the Data Handling Committee.

Relevant concomitant care permitted or prohibited during the trial. In principle, no new drugs should be added during the observation period, or drugs in use at the time of obtaining consent should be discontinued, switched, or the dose changed. In principle, no new drugs should be added during the observation period, or drugs in use at the time of obtaining consent should be discontinued, switched, or the dose changed.

Sample size. This case design study will be exploratory in nature. The feasibility of the target number of cases to be enrolled is based on the number of cases in previous studies.[17,18]

Outcomes. The primary outcome of this study will be the change in GSRS score from objective 1 to objective 2. Secondary outcomes are as follows: (1) change in other GSRS scores, Bristol

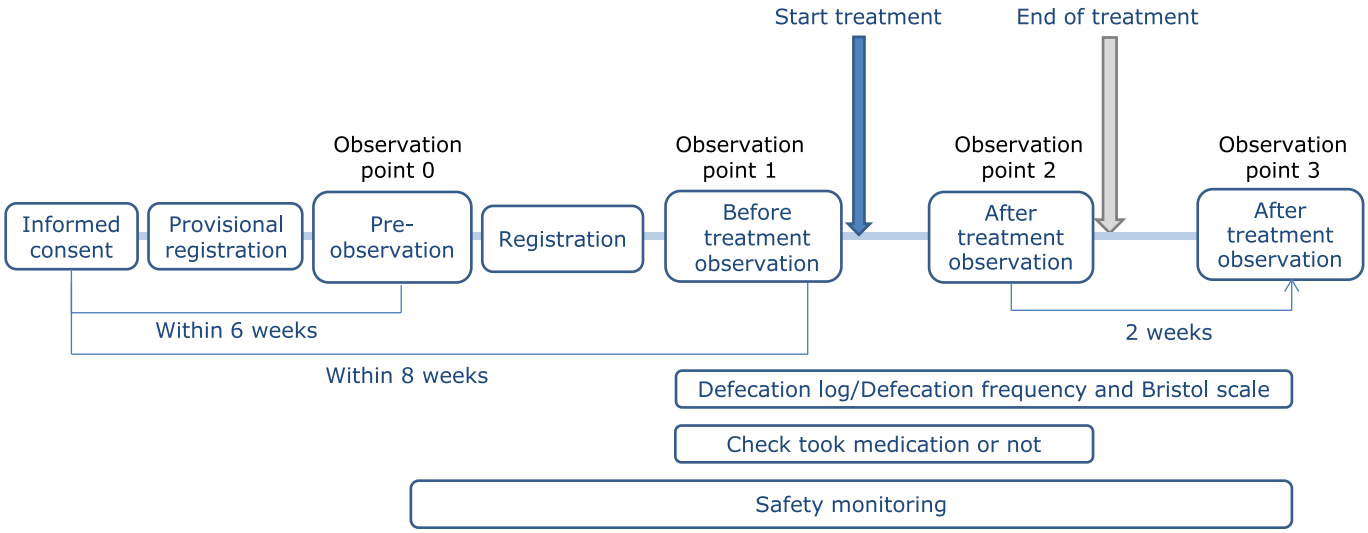

Fig. 1. Study design of Big STAR study.

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Stool Scale score, HbA1c level, glycoalbumin level, fasting plasma glucose level, C-peptide level, active GLP-1 level, total bile acid level, body mass index (BMI), blood pressure, and gut microbiota (type of enterobacteria, relative abundance, alpha diversity, and beta diversity) from objective 1 to objective 2; (2) change in GSRS score from objective 1 to objective 2, after adjusting for age, sex, BMI, type of gastrointestinal symptoms, and administered drugs; and (3) correlations between changes in fecal properties and changes in abovementioned biomarkers.

All adverse events that occur during the intervention period must be reported, irrespective of their suspected causal relationship to the study drug. They must be followed until they have disappeared or for at least 2 weeks after completion of the study period (after discontinuation) and will be assessed with respect to insulin type, onset, outcome, severity, and causal relationship of the study drug.

Provisions for post-trial care. Since this study was conducted under the normal medical practice, in principle, no special compensation will be provided in the event of health damage caused by the drugs under study. It is treated in exactly the same way as health damage and medical accidents that occur during normal medical treatment, and in the case of liability, it will be dealt with by doctors' liability. Such compensation is covered by the Pharmaceutical Side Effect Damage Relief System of the Pharmaceuticals and Medical Devices Agency or by the Product Liability Law and Product Liability Insurance. However, since the study design is an intervention study, to handle with the event of health damage resulting from non-medical activities, we obtain clinical research insurance.

Data Collection and Management

Plans for assessment and collection of outcomes. Data will be gathered by researchers using a central registration number assigned by the data center. The data management operations of the study will be performed by the data management personnel in accordance with the procedures.

Confidentiality. Information that can identify research subjects (name, address, telephone number, etc.) is not entered into the data at the time of registration or recorded in the case report form by persons outside the medical institution where the research is conducted. The central registration number is used by the data management staff when referring to the performing medical institution. The researcher identifies (anonymizes) the research subjects using the research subject identification control tables (correspondence tables) that he or she maintains.

Statistical methods. A full analysis set (FAS) analysis, per-protocol set (PPS) analysis, and safety analysis will be performed for the primary and secondary endpoints. A p value <5% will be considered statically significant.

The FAS group will consist of all study populations enrolled in this study, excluding participants for whom written informed consent will not be obtained or those registered outside the study period. The PPS group will include all participants who were excluded from the FAS group, who violated inclusion or exclusion criteria, who were administered prohibited drugs, or whose medication adherence was ≥120% or <75%. The safety analysis group will consist of participants who were enrolled in the study and received some or all study interventions.

Categorical variables will be expressed as frequencies and percentages, and continuous variables will be expressed as the numbers of cases, averages, SDs, medians, minimums, and maximum values. The primary and secondary endpoints will be evaluated using the paired t test or Student's t test. Non-normally distributed values will be analyzed after logarithmic transformation or using the Wilcoxon signed-rank test. Correlations will be evaluated using Spearman's rank correlation coefficient. The safety analysis will consist of a list of all adverse events.

Oversight and monitoring. Researchers will conduct safety monitoring of research subjects throughout the research period, including the occurrence of diseases and adverse events.

The monitoring operations of the research will be carried out by the person in charge of monitoring who is independent from investigators in accordance with the monitoring procedures.

Before enrollment of the first study subjects begins, the research plan will be registered and published in jRCT, a database maintained by the Ministry of Health, Labour and Welfare. The progress of the research will be updated in a timely manner and the completion of the research will be reported without delay. All data and results obtained from this study are the property of the investigating physician. The results will be published in scientific journals by the investigators.

Discussion

This study explores the effect of the probiotic BBG9-1 on changes in the symptoms of constipation or diarrhea in patients with T2D who use metformin.

Metformin, an insulin sensitizer, is a common first-line agent for managing hyperglycemia in patients with T2D.[12] However, its use is associated with an increased risk of gastrointestinal complications.[5] In fact, according to the medical package insert, 40.5% of patients who use metformin exhibit diarrhea, and >1% have constipation.[19] Thus, many patients discontinue metformin use. In contrast, a recent study revealed that probiotics containing BBG9-1 improve the balance of intestinal flora and result in improved defecation in the animal model using metformin.[16] Thus, there is a possibility that using probiotic BBG9-1 can ease the symptoms of constipation or diarrhea in patients with T2D who use metformin.

This is the first study to investigate the effect of probiotic BBG9-1 on change in the symptoms of constipation or diarrhea in patients with T2D who use metformin. The results of our study provide the usefulness of probiotic BBG9-1 in constipation or diarrhea in patients with T2D who use metformin.

Author Contributions

YH led the drafting of the manuscript. HN, SH, and MF reviewed the manuscript and study design and contributed to the final draft. The other authors will recruit participants and contributed to the final draft.

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Abbreviations

BMI	body mass index
FAS	full analysis set
GLP-1	glucagon-like peptide-1
GSRS	Gastrointestinal Symptom Rating Scale
PPS	per-protocol set
T2D	type 2 diabetes

Conflict of Interest

YH reports grant from Asahi Kasei Pharma and personal fees from Daiichi Sankyo Co. Ltd., Mitsubishi Tanabe Pharma Corp., Sanofi K.K., and Novo Nordisk Pharma Ltd., outside the submitted work. TO reports grants from Combi Corp., and personal fees from MSD K.K., Sumitomo Dainippon Pharma Co., Ltd., Novo Nordisk Pharma Ltd., Daiichi Sankyo Co. Ltd., Eli Lilly Japan K.K, Takeda Pharma Co. Ltd., Nippon Boehringer

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Trial Status

This study started Feb 19, 2020 and protocol ver. 1.2. The recruitment will be completed at Sep 30, 2020.

Declarations

Ethics approval and consent to participate. This study was registered with the Japan Registry of Clinical Trials (jRCTs051190109) and has been approved by the ethics committees of the Kyoto Prefectural University of Medicine (CRB5180001). This study is to be conducted according to the Declaration of Helsinki. Written informed consent will be obtained from all the participants.

Consent for publication. Not applicable.

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Availability of data and materials. Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Authors' information. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship of this manuscript, take responsibility for the integrity of the work as a whole, and have given final approval for the version to be published.

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