Gut microbiota specifically mediates the anti-hypercholesterolemic effect of berberine (BBR) and facilitates to predict BBR's cholesterol-decreasing efficacy in patients

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Highlights

- The gut microbiota is necessary for the lipid-lowering effect of BBR.
- The BBR-conditioned gut microbes effectively ameliorate hyperlipidemia.
- Blautia is indispensable for the hypercholesterolemia-alleviating effect of BBR.
- The cholesterol-lowering effect of BBR was closely related to the gut microbiota in human beings.
- The baseline abundance of Alistipes and Blautia is effective to predict the cholesterol-lowering efficiency of BBR.

Abstract

Introduction: Gut microbiota has been implicated in the pharmacological activities of many natural products. As an effective hypolipidemic agent, berberine (BBR)'s clinical application is greatly impeded by the obvious inter-individual response variation. To date, little evidence exists on the causality between gut microbes and its therapeutic effects, and the linkage of bacteria alterations to the inter-individual response variation.

Objectives: This study aims to confirm the causal role of the gut microbiota in BBR's anti-hyperlipidemic effect and identify key bacteria that can predict its effectiveness.

Methods: The correlation between gut microbiota and BBR's inter-individual response variation was studied in hyperlipidemic patients. The causal role of gut microbes in BBR's anti-hyperlipidemic effects was subsequently assessed by altered administration routes, co-treatment with antibiotics, fecal microbiota transplantation, and metagenomic analysis.

Abbreviations: AMPK, AMP-activated protein kinase; BBR, berberine; HFD, high-fat diet; H&E, Hematoxylin and Eosin; InsR, insulin receptor; LDL-c, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptors; PS, the responsive subjects; NPS, the non-responsive subjects; RF analysis, Random forest analysis; ROC, receiving operating characteristic; SCFAs, short-chain fatty acids; TC, total cholesterol; TG, triglycerides.

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Introduction

Compelling evidence has revealed that gut microbiota contributes to the exertion of the pharmacological activities of various drugs and active substances, such as metformin [1], cranberry [2], ganoderma [3], and Pandanus tectorius [4]. Therefore, understanding how gut microbes participate in human health and therapeutics has significant consequences for treating disease and minimizing adverse effects in clinical research. Nowadays, although considerable data have been published on drug–gut microbiota association, the causality between gut microbes and therapeutic effects as well as the key causal taxa are rarely explored.

In clinical practice, patients usually manifest distinct responses to the same drug, which is called inter-individual response variation/difference [5,6]. For instance, more than 20% of type 2 diabetic patients do not respond to or tolerate metformin [7], and the hyperlipidemic patients with familial chylomicronemia or combined dyslipidemia are partially responsive or even unresponsive to statins [8]. The unresponsiveness to certain drugs not only deprives patients of effective disease management but also greatly impedes the development of new drugs. Besides the well-documented factors such as genetics, dietary habit, and psychology [9,10], recent investigations have demonstrated gut microbiota is emerging as another determinant for inter-individual response difference [11,12]. It has been widely accepted that gut microbiome play essential roles in regulating host physiological processes and drug metabolism [13], and their composition varies greatly in population [14], thus probably leading to the different response to the same medical agent. An increased abundance of Prevotella copri may result in unresponsiveness to the hypoglycemic effect of metformin [15], and diminishment of gut microbes by antibiotics substantially deteriorates the lipid-lowering effect of rosuvastatin [16]. A recent study indicated that targeted inhibition of gut bacterial β-glucuronidase activity markedly enhances the anticancer efficacy of irinotecan [17]. Hence, classifying the correlations between gut bacteria and drug responsiveness will shed new light into understanding the mechanism of response variation and may provide potential solutions to improve the efficacy of medical agents.

Berberine (BBR), a natural plant alkaloid extracted from Berberis aristata and Coptis chinensis (Huanglian), has been reported to be an excellent natural remedy for metabolic disorders, including hyperlipidemia [18,19]. Multiple potential mechanisms have been proposed regarding its pharmacological actions, such as upregulation of low-density lipoprotein receptors (LDLR) in liver [18], activation of AMP-activated protein kinase (AMPK) in adipose tissue and muscles [20,21], and stimulation of insulin receptor (InsR) [22,23]. However, the oral bioavailability of BBR is rather inadequate and barely reaches the effective level at the target sites when orally administered [24]. Recent investigations have demonstrated that the anti-hyperlipidemic effect of BBR is closely related to its modulation on gut microbiota. Still, there is no solid evidence to certificate the causality between gut microbes and BBR’s beneficial functions. Moreover, the distinct and even opposite responses occur among patients taking BBR administration [18], largely hindering its’ general clinical application.

In the current study, we conducted a 3-month clinical study on the anti-hyperlipidemic effect of BBR, which not only confirmed its therapeutic effect but also revealed its obvious inter-individual response variation. We further performed metagenomic analysis between responsive and non-responsive patients, which linked the cholesterol-lowering of BBR to the modulation of the gut microbiota and displayed that the baseline levels of some gut bacterial taxa could precisely predict the efficacy of BBR against hypercholesterolemia. We then performed causality experiments in mice by altered administration routes, concomitantly treatment with antibiotics and fecal transplantation, which not only confirm the causal role of gut microbes in the lipid-lowering effect of BBR, but also identified Blautia as a key taxon that conveyed BBR’s anti-hypercholesterolemic action in both animals and human beings.

Methods

Clinical trial

A randomized, double-blind, placebo-controlled clinical trial with a treatment period of twelve weeks was carried out at Dongzhimen Hospital (Beijing, China). Eighty-three hyperlipidemic patients were recruited between April 2019 and January 2020. The eligibility criteria and exclusion criteria were provided as supplemental data. All patients were randomly assigned to receive double-blind berberine (0.5 g, twice daily) or placebo prepared in indistinguishable tablets. The BBR and placebo tablets were produced by the same pharmaceutical company (Shanxi Zhendong Pharmaceutical Co., Ltd. Lot NO:20190301). The participants in both groups also received lifestyle interventions included regulation of the diet and exercise. Participants will be asked to be strict about their diet and to avoid short-term or high-intensity exercise. Risk factors, such as smoking and alcoholics, will be controlled strictly. Researchers will emphasize the importance of diet and exercise to subjects at each treatment visit.

Patients visits were done between 0700 and 0800 am after an overnight fast. Biochemical measurements of serum lipids were performed in the clinical laboratory of Dongzhimen Hospital. Serum lipids were measured at 0, 4th, 8th, and 12th week. The participants will be asked “which type of stool they have” at each visit during the treatment period. The stool samples were obtained from each patient at the 0 and 12th week.

Animal experiment for necessity analysis

As male animals are more stable for metabolism study and most previous investigations on BBR’s pharmacological activity and its
modulation on gut microbiota were carried out on male rodents [25], we chose male mice in this study to match our results with prior reports. Eight-week-old male ICR mice were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). After acclimatized for one week on a normal chow diet, mice were fed for five weeks with a normal chow diet which contains 45% fat, 50% carbohydrate, and 22% protein with a total calorific value of 6.095 kcal/kg (Chow group) or high-fat diet (HFD) which contains 22% fat, 35% carbohydrate, and 21% protein with a total calorific value of 10.516 kcal/kg (HFD group and other test groups). The Chow and HFD groups were given distilled water only. Animals on HFD were orally (po, 200 mg/kg per day, HFD + BBRp) or intraperitoneally (ip, 6 mg/kg per day, HFD + BBRp) administrated with BBR, or gavaged with BBR (200 mg/kg per day) and antibiotics (ampicillin + norfloxacin, 300 mg/kg per day respectively) (HFD + Abs + BBRp), or gavaged with BBR (200 mg/kg per day) and antibiotics (ampicillin + norfloxacin, 300 mg/kg per day respectively) (HFD + Abs + BBRp), with antibiotic-only group [HFD + Abs] as control. As the oral bioavailability of BBR is < 1% [26], the intraperitoneal dose of BBR in this study is about three times of that obtained by 200 mg/kg po. Stools for metagenomic analysis were acquired daily for the subsequent 28 days. Feces from each animal (200 mg/L for each animal) by oral gavage for four weeks, were pretreated with antibiotics cocktail (ampicillin + norfloxacin, 300 mg/kg per day) for five days to eliminate gut bacteria, then they were fed with HFD and inoculated daily with fresh fecal microbial suspension. Male recipient mice (eight mice per group) were pretreated with antibiotics cocktail (ampicillin + norfloxacin, 300 mg/kg per day) for five days to eliminate gut bacteria, then they were fed with HFD and inoculated daily with fresh fecal microbial suspension. Microbiota analysis. The detailed procedure of fecal microbiota analysis was described in the supplementary methods. We obtained the initial (week0) and final (week12) fecal samples from 51 patients (28 in BBR group and 23 in placebo group) and analyzed their gut microbiome composition by shotgun sequencing metagenomics. The impact of BBR on the overall structure of gut microbiota was assessed by community alpha diversity (Chao1 index indicates community taxon richness, Shannon index indicates community diversity, and Simpson index shows equivalency of each microbe) and community variation (principal coordinate analysis (PCoA)). Three-month treatment of BBR has only minimal impact on alpha diversity and the overall structure of gut microbiota in our hyperlipidemic cohort (Fig. S1), indicating community diversity, and the Simpson index shows equivalency of each microbe. Moreover, BBR did not significantly decline serum TG until treatment for 12 weeks. In contrast, it steadily decreased serum TC and LDL-c from the first month after medication (Fig. 1). These results indicated that BBR preferred to mitigating hypercholesterolemia. Importantly, we found that a large portion of patients manifested the decreased serum lipids level after BBR treatment, but some subjects were less responsive to BBR treatment, especially for intervention on serum TC and LDL-c (Fig. S1), exhibiting an obvious inter-individual variation in the therapeutic efficiency of BBR. Gut microbiota tightly associated with the therapeutic efficacy of BBR on cholesterol not triglyceride We obtained the initial (week0) and final (week12) fecal samples from 51 patients (28 in BBR group and 23 in placebo group) and analyzed their gut microbiome composition by shotgun sequencing metagenomics. The impact of BBR on the overall structure of gut microbiota was assessed by community alpha diversity (Chao1 index indicates community taxon richness, Shannon index indicates community diversity, and Simpson index shows equivalency of each microbe) and community variation (principal coordinate analysis (PCoA)). Three-month treatment of BBR has only minimal impact on alpha diversity and the overall structure of gut microbiota in our hyperlipidemic cohort (Fig. S2), possibly due to an adaptation of gut microbes to BBR. We further correlated the TC/TG-decreasing efficacy of BBR with gut microbiota to verify whether the response difference to BBR treatment attributes to the modulation of gut microbiota. For patients with hypertriglyceridemia, the overall structure and
alpha diversity of gut microbiota showed no alterations between
the responsive (PS, whose serum TG decreased after treatment)
and the non-responsive (NPS) subjects (Fig. S3A-E), along with
the almost identical profiles of key genera abundance (Fig. S3F).
These findings pointed out a conjecture that the TG-decreasing
effect of BBR hardly relates to its regulation on gut microbial com-
Community. On the contrary, gut microbes exhibited a significant
\( p < 0.05 \) adonis separation between the PS and the NPS patients
towards BBR’s cholesterol-decreasing effect (Fig. 2A-C). The alpha
diversity of microbiome in the PS subjects was increased after
BBR treatment, while the NPS ones showed some decrease
(Fig. 2D). Therefore, these results collectively suggested that mod-
ulation of BBR on gut microbiota might specifically contribute to its
cholesterol-decreasing effect.

The baseline levels of Alistipes and Blautia promisingly predict the
cholesterol-lowering function of BBR

Next, we focused on the taxa that were markedly different
between the PS and NPS patients in their initial gut microbiota,
aiming to find key bacteria possessing potentials to predict the
therapeutic efficacy of BBR before medication. Among all the main
genera, only Alistipes was significantly different in the baseline
decal bacterial community between PS and NPS patients (Fig. 3A).
At the species level, A. putredinis, A. shahii and A. finegoldii were
the major Alistipes members that were prominently higher in
NPS patients in comparison with the PS subjects (Fig. 3B). Co-
abundant network analysis revealed that three species of genus
Blautia, that were, Ruminococcus gnavus, R. torques and R. obeum,
were significantly negative to the Alistipes members (Fig. 3C). Ran-
dom forest analysis also showed that A. finegoldii, A. shahii, A. seneg-
galsis and R. gnavus were among the top 15 species that
contributed the difference in gut microbiome between PS and
NPS subjects (Fig. 3D). In light of the critical roles of Alistipes and
Blautia in the lipid-lowering effect of BBR [29], we investigated
their predictive ability by ROC analysis and found that the baseline
abundance of these two genera could effectively predict the
cholesterol-decreasing efficacy of BBR with AUC of 0.903 (95% con-
fidence interval 0.761–1.000, \( p = 0.007 \)) (Fig. 3E).

Taking this prediction model, we selected 11 persons from the
18 hypercholesterolemic patients in our cohort as predicated BBR
responders in which 90.91% (10/11) patients were actual respon-
ders, markedly higher than the total responsive rate of BBR
(66.67%, 12/18). These predicted responders showed a significant
decline in serum TC (\( P = 0.002 \)) as compared to the placebo-
treated subjects. Meanwhile, they were also more responsive to
BBR’s TG-decreasing (\( -1.38 \) vs. \( -0.70 \)) and LDL-c-decreasing
(\( -0.55 \) vs. \( -0.31 \)) effects than all hypercholesterolemic patients
that received BBR medication (Fig. S4A). For the entire cohort that
includes both hypercholesterolemic and hypertriglyceridemic

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**Fig. 1.** BBR effectively decreases blood lipids in hyperlipidemic patients. Eighty-three hyperlipidemic patients were treated with BBR (42 subjects, 1 g/day) or placebo (41 subjects) for 3 months. The lipids changes at each time point compared to the baseline levels were analyzed.
patients, 18 subjects were predicted as BBR responders by our predict model in which 16 (88.89%) patients were responsive to BBR’s anti-hypercholesterolemic or anti-hypertriglyceridemic efficacy, while the overall effectiveness of BBR in this term was 82.14%. These predicted responders were also more responsive to BBR’s TC-decreasing (\(\frac{C_0}{C_0} = 0.62\) vs. \(\frac{C_0}{C_0} = 0.28\)) and TG-decreasing (\(\frac{C_0}{C_0} = 1.21\) vs. \(\frac{C_0}{C_0} = 0.93\)) effects than the whole cohort (Fig. S4B).

Gut microbiota is important for the lipid-lowering effects of BBR

To confirm the causal role of the gut microbiome in the anti-hyperlipidemic effects of BBR, we further set up paired experimental groups of mice treated with oral (HFD + BBRpo, 200 mg/kg) or intraperitoneal (HFD + BBrip, 6 mg/kg) administration of BBR, as well as groups with oral administration of BBR and antibiotics (HFD + BBrip + Abs; ampicillin and norfloxacin [Abs], 300 mg/kg each), with an antibiotic-only group (HFD + Abs) as control. Orally administration of BBR significantly alleviates the adverse effects of HFD on obesity, lipid and glucose profile, insulin sensitivity, and liver steatosis (Fig. 4; Fig. S5). However, these beneficial effects were markedly reduced when BBR was administered via intraperitoneal injection (HFD + BBrip vs. HFD + BBripo; Fig. 4; Fig. S5), indicating that the lipid-lowering effect of BBR is not solely achieved by its entry into the circulation. When combined with antibiotics, the therapeutic effects of BBripo were nearly disappeared (HFD + BBrip + Abs vs. HFD + Abs; Fig. 4; Fig. S5), implying that gut micro-

Fig. 2. The cholesterol-lowering efficiency of BBR is closely related with its modulation on gut microbiota. A-B. The principal coordinate analysis (PCoA) (A) and nonmetric multi-dimensional scaling (NMDS) analysis (B) of gut microbiota. (C) The Bray-Curtis distance-based clustering analysis. (D) The changes of alpha diversity indices compared to the baseline values. We obtained the initial (week0) and final (week12) fecal samples from 51 patients (28 in BBR group and 23 in placebo group) and analyzed their gut microbiota composition by shotgun sequencing metagenomics. *P < 0.05.
Fig. 3. The baseline abundance of Alistipes and Blautia spp. are effective to predict the cholesterol-lowering efficiency of BBR in hyperlipidemic patients. (A) The genus profile of responsive (PS) and non-responsive (NPS) patients, and Alistipes is the only dominant genus whose baseline abundance is significantly different between PS and NPS patients. (B) The species profile of PS and NPS patients, and three Alistipes spp. are significantly different between PS and NPS patients at the baseline level. (C) Co-occurrence network established by SparCC analysis. The area of each node indicates the accumulated abundance of the species, and the portion of each group was displayed in different colours. The connecting edges indicate positive (orange) or negative (blue) correlations between species. (D) The top 15 species that discriminate the PS and NPS patients based on random forest analysis. (E) Receiver operating characteristic curve (ROC) for the combination of Alistipes and Blautia spp. Area under curve (AUC) and the 95% confidence interval are also shown.
Fig. 4. Parenteral administration or antibiotic treatment largely weakens BBR’s lipid-lowering effect. (A) Serum lipids and glucose levels in each treatment group; error bars denote standard error in measurements. (B) Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT) in each group. (C) Hematoxylin and eosin (H&E) staining of the liver (bar = 10 μm). (D) Steatosis score of different treatment groups; each dot represents a liver sample wherein steatosis was diagnosed. (0–3) was evaluated as follows: 0, no involvement; 1, mild involvement; 2, moderate involvement; and 3, severe involvement. E-F Liver total cholesterol (TC) (E) and triglyceride (F) measurements in different treatment groups. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, N.S. = non-significant.
biota may play crucial roles in mediating the anti-hyperlipidemic effect of BBR.

We observed consistent shifts in the fecal microbiome within each treatment group. The microbiome of BBRip mice was closest to that of those on the HFD diet alone, and oral administration of BBR and antibiotics markedly shifted the structure of microbial communities (p < 0.001 in adonis test; Fig. 6A-C). Similar in human beings, oral gavage of BBR significantly enriched Blautia and decreased the abundance of Alistipes (Table S2). Besides, BBR also increased the population of bacteria belonging to Akkermansia, Clostridium XI, Robinsonella, Cronobacter, Anaerostipes and Coprobacillus, and decreased the abundance of Helicobacter, Enterorhabdus and Desulfovibrio (Table S2). BBRpo mice showed a different microbial community structure compared with mice treated with only antibiotics, and combination treatment with antibiotics substantially diminished the modulation of the microbiota by BBR (Fig. 6D), thereby exhibiting a distinct impact on gut microbiota between BBR and antibiotics.

BBR-modulated gut microbiota prominently ameliorates hyperlipidemia

To figure out whether the shifts in the microbiome confer the beneficial effects of BBR, or just occur secondary to BBR treatment, we undertook fecal transplantation from donor mice with oral-administration of BBR to mice with an HFD feeding and found that fecal transplantation indeed induced a beneficial influence with the similar extent to that observed in BBRpo mice. Specifically, the transfer of the microbiome resulted in less body and fat weight (Fig. 5A-E), lower serum lipids and glucose levels (Fig. 5F),

Fig. 5. Fecal material transplantation after BBR treatment prevents HFD-induced hyperlipidemia as effectively as BBR. (A–D) Bodyweight (A), bodyweight gain (B) and the weights of liver (C), subcutaneous fat (D) and epididymal fat (E) of different treatment groups, respectively. (F) Serum levels of TC, TG, LDL-c, and glucose. (G) Oral glucose tolerance test (OGTT) and insulin tolerance test (ITT). (H–J) H&E staining of liver (bar = 10 μm) (H) and hepatic levels of total cholesterol (TC)(I), and triglycerides (TG) (J) from different treatment groups, respectively. (K) HPLC for BBR in BBR soup (bottom panel) and the collected fecal material after BBR administration (top panel), indicating the absence of BBR in fecal materials used for the transplant. *P < 0.05, **P < 0.01, ***P < 0.001, N.S. = non-significant.
improved oral glucose tolerance (Fig. 5G), and alleviation of liver steatosis (Fig. 5H-J). Given the undetectable level of BBR in fecal material (Fig. 5K), our results suggested that BBR-modulated microbiota holds great potential to improve hyperlipidemia. Further detailed analysis of the microbiota displayed that the fecal microbiota transplant significantly shifted gut microbes (Fig. S7A-B) and promoted the growth of short-chain fatty acids (SCFAs)-producing taxa, which have been intensively reported to regulate host lipid metabolism, including *Clostridium XI*, *Anaerostipes*, *Blautia*, *Akkermansia*, and *Coprobacillus*, and decreased the abundance of *Alistipes*, *Helicobacter*, *Enterorhabdus* and *Desulfovibrio* (Fig. S7C-D; Table S3). Again, *Alistipes* and *Blautia* were similarly modulated by BBR in mice and in human beings, suggesting a universal role of these two genera in the lipid-decreasing action of BBR.

**Dysregulation of Blautia impaired the cholesterol-lowering action of BBR**

In both necessity (changes in administration routes or concomitant treatment with antibiotics) and causality (fecal transplantation) experiments, thirteen genera with relative abundance above 0.01% were coordinately altered by BBR or BBR-modulated gut microbiota (Table S3). The *Blautia*, *Akkermansia*, *Robinsoniella*, *Anaerostipes* and *Coprobacillus* were significantly enriched, while *Alistipes*, *Helicobacter* and *Enterorhabdus* were largely decreased by oral administration of BBR or BBR-modulated microbes (Fig. S8). According to these findings, we speculated that these genera might be necessary and causative for BBR’s lipid-reducing actions.

Little feasible method is currently available to precisely diminish certain bacterial genus. In light of the different individual responsiveness to berberine in human beings, we speculate that it may be the same in animals and repeated experiments several times to come across the situation where BBR failed to regulate the serum lipids. Intriguingly, BBR was found in one experiment to merely preserve the anti-obesity and anti-hypertriglyceridemia effects (Fig. 6A-C), while the anti-hypercholesterolemia efficacy was substantially diminished (Fig. 6C). Subsequently, we analyzed the profile of the above-mentioned genera that were tightly-related to BBR’s therapeutic roles. The modulations of BBR on *Alistipes*, *Helicobacter*, *Enterorhabdus*, *Akkermansia*, *Robinsoniella* and *Coprobacillus* were all preserved, whereas the elevation of *Blautia* was disappeared in this experiment (Fig. 6D). These results provided a hint that *Blautia* might be a key taxon to convey BBR’s cholesterol-decreasing effect.

**Discussion**

Accumulating researches have implicated that gut microbiota widely participates in the exertion of various drugs’ therapeutic efficacies, for instance, berberine (BBR) [30]. Previous studies revealed that the hyperlipidemia-alleviating effect of BBR was mediated by the gut microbiota [31–34]. However, to date, the causality between specific gut microbes and BBR’s pharmacological effects remains to be verified. Meanwhile, BBR medication usually exhibits an obvious inter-individual variation [18], thus heavily impeding its clinical application. In the present study, we not only demonstrated the necessity and sufficiency of gut microbes in the...
lipid-lowering effect of BBR in mice model, but also provided a first evidence that the commensal genus, Blautia, is indispensable for BBR’s anti-hypercholesterolemic efficacy. BBR medication in patients further corroborated that the modulation of BBR on gut microbiota was closely related to its cholesterol-lowering action. Particularly, the baseline levels of Alistipes and Blautia could predict the anti-hypercholesterolemic efficiency of BBR, possibly facilitating to improve the overall effectiveness of BBR and achieve the ultimate clinical therapeutics.

BBR is an excellent natural lipid-lowering drug, yet with a very poor oral availability. Many researchers have noticed the remarkable influence of BBR on gut microbiota [1,2,33,35–37] and proposed that the modulation on gut microbiota may be a main mechanism underpinning its anti-hyperlipidemic action. Unfortunately, most studies only demonstrated that changes of gut microbiome were synergistic with BBR administration [3], without offering sufficient evidence towards the causality between bacteria and its beneficial effects. In this study, we first introduced different administration routes and found that intraperitoneal injection of BBR (BBRip) exerted a weaker efficiency to ameliorate hyperlipidemia than oral administration (BBRpo), though the biological availability of BBR was increased by over three times in BBRip mice (Fig. 4), suggesting that oral bioavailability is not the sole factor responsible for BBR’s therapeutic efficacy. We further performed experiments in antibiotics-induced pseudo-germfree mice which revealed that elimination of gut microbes substantially abolished BBR’s lipid-lowering efficiency (Fig. 4), clearly pointing out the necessity of gut microbiota. Moreover, the benefits of BBR could be well-transferred by fecal microbiota transplantation from BBRpo-treated mice to HFD mice (Fig. 5), again corroborating the critical roles of gut microbiome. Collectively, our findings demonstrate that modulation of gut microbiota is both necessary and sufficient to mediate the anti-hyperlipidemic effect of BBR, ruling out the possibility of microbiome shifts being merely secondary.

For modulation on microbial structure, several gut bacteria including Akkermansia, the SCFA-producing and bile salt-metabolizing germs have been previously suggested as key taxa closely-associating with the pharmacological functions of BBR [33,36,38–40], yet none of them has been certified regarding its necessity by experimental evidence. Our association analysis showed that the enrichment of Blautia, Akkermansia, Robinsoniella, Coprobcacillus and Anaerostipes and the decrease of Alistipes, Heliocobacter, and Enterorhabdus were tightly related to BBR beneficial effects in both necessity (administration route alterations and antibiotics treatment) and sufficiency (fecal microbiota transplantation) experiments (Fig. S8). Interestingly, we found in one experiment where oral administration of BBR only retained the impacts on bodyweight and TG, but substantially lost its TC- and LDL-c-alleviating actions (Fig. 6A–C). Correspondingly, the detailed taxonomic analysis displayed that modulations of BBR on Akkermansia, Robinsoniella, Coprobcacillus, Anaerostipes, Alistipes, Heliocobacter, and Enterorhabdus were preserved, whereas the growth of Blautia was apparently dysregulated (Fig. 6D). Based on these, we speculated that Blautia might function as an important genus contributing to the cholesterol-decreasing effect of BBR. Indeed, two recent studies have claimed that this genus associates with visceral fat accumulation in young adults [41] and with the therapeutic efficacy of a traditional Chinese herbal formula containing BBR [29]. Hence, our results provided the first evidence that Blautia is a causal taxon responsible for the cholesterol-decreasing function of BBR.

Another important finding in this work is that gut microbiota involves in the inter-individual response variation of BBR medication. To investigate the association of BBR’s therapeutic efficacy with gut microbiome in human, we conducted a clinical research on recruited 83 hyperlipidemic patients. Although BBR could effectively decrease serum TC, TG and LDL-c levels as compared to placebo, about 21.43% (9/42), 38.10% (16/42) and 54.76% (23/42) patients were still not responsive to the TG-, TC- and LDL-c-decreasing effects of BBR, respectively (Fig. S1), showing an obvious inter-individual response variation. Up till now over twenty clinical studies have been published on the hyperlipidema-ameliorating effect of BBR [42], but the consequent varied responses are scarcely investigated. Given the prominent modulation of BBR on gut microbes in mice, we conjectured that the versatile commensal bacteria would actively participate in the annoying responses in patients. To figure out this, we then correlated BBR’s TC/TG-lowering efficacy with its regulation on microbiota. 3-month medication of BBR did not significantly change the overall structure of gut microbiota (Fig. S2), which might reflect an adaptive response of gut microbes to chronic therapeutics. However, more detailed analysis unraveled that the cholesterol- not triglyceride-decreasing efficacy of BBR was intimately related to the apparent alterations of microbial community (Fig. 2, Fig. S3), providing an important evidence that the hypercholesterolemia-mitigating effect of BBR may involve the function of gut microbes.

An essential aim of precision medicine is to find right drugs for a particular patient to achieve satisfactory personalized medication [43]. From the point of view of medicine, finding suitable patients for a specific drug is also an effective way to fulfill the precision therapy. In light of the strong association between BBR’s TC-decreasing efficiency and gut microbiota, we attempted to uncover certain bacteria with a prediction ability towards BBR’s efficacy. Intriguingly, we found that higher baseline abundance of Alistipes spp. and Blautia spp. could predict the effective response in patients [43A–B]. However, further analysis further indicated that the baseline abundance of Alistipes and Blautia in initial gut microbiota could promisingly predict the effective- ness of subsequent BBR medication against hypercholesterolemia (Fig. S3D). Tong et al. also raised that alterations of Alistipes (decrease) and Blautia (increase) associate with the alleviation in hyperlipidemia of a traditional Chinese herbal formula [29]. These findings inspire us to depict a vision where the personalized responses to BBR treatment could be conveniently predicted just by quantifying baseline levels of certain genera (e.g. Alistipes and Blautia) in a painless, time- and cost-saving manner before actual medication. Moreover, manipulation of Alistipes and Blautia amid BBR medication might be a potential method to improve the overall efficacy of BBR, ultimately promoting its clinical-scale application.

Conclusions

In conclusion, our studies first demonstrate that gut microbiota is not only necessary but also sufficient to mediate the lipid-lowering effect of BBR and identify Blautia to be a critical commensal genus to convey its anti-hypercholesterolemic action in mice. Furthermore, the cholesterol- not triglyceride-lowering efficacy of BBR in patients intimately associates with its modulation on gut microbiota, and the baseline levels of Alistipes and Blautia could precisely predict the efficacy of BBR against hypercholesterolemia, thereby holding promising potential to tackle its inter-individual response variation and fulfill personalized medication.

Compliance with ethics requirements

All the animal experiments were performed in accordance with the National Institutes of Health regulations for the care and use of animals in research. The protocol was approved by the medical...
Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Availability of data and material

The sequencing data is deposited at NCBI archive with project numbers PRJNA557542 for antibiotics and fecal transplantation study; PRJNA5599041 for Blautia spp. absence study. Other information can be presented on request. All data relevant to the study are included in the article or uploaded as supplementary information.

Appendix A. Supplementary material

Additional file 1: Additional methods and Supplementary Figures S1-S8 and Table S1-S3. Supplementary data to this article can be found online at https://doi.org/10.1016/j.jare.2021.07.011.

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