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Original Article

Berberine alleviates chronic inflammation of mouse model of type 2 diabetes by adjusting intestinal microbes and inhibiting TLR4 signaling pathway

Weiliang Cao[1*], Li Hu[1*], Hu Chen[2], Yongqiang Gao[1], Yu Liang[1], Yuan Wu[2], Qinglan Yang[1], Na Tang[1], Jinsong Cao[2], Jianhua Xiao[1]

[1]*Institute of Pathogenic Biology, Medical College, University of South China, Hengyang, Hunan Province, China;* [2]*Clinical Laboratory, The Second Affiliated Hospital of University of South China, Hengyang, Hunan Province, China.* [*]*Equal contributors.*

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Abstract: Background: Type 2 diabetes mellitus (T2DM) is associated with systemic chronic inflammation induced by gut microbes imbalance. Although it was revealed that Berberine (BBR) can decrease fasting plasma glucose (FPG) in T2DM patients, it is rarely absorbed in gastrointestinal tract so that the hypoglycemic mechanism is unclear. Methods: We established mouse models of T2DM and treatment the animals with BBR. Meanwhile, the levels of FPG and fasting insulin (FIns), changes of intestinal microbes, mRNA levels of TLR4, TNF-α, IL-1β and IL-6, pathological damage and macrophages (MΦs) infiltration in pancreatic islets of mice were measured. Furthermore, THP-1 derived MΦs were used to study the influence of BBR to LPS-TLR4 axis. Results: FPG, FIns, and mRNA levels of TLR4, TNF-α, IL-1β and IL-6, pathological damage and MΦs infiltration were significantly increased in pancreatic islets of T2DM mice and the probiotics obviously decreased in intestinal tract compared with normal control. However, these parameters were markedly decreased and the probiotics obviously increased in intestinal tract after administration of BBR. Moreover, in THP-1 derived MΦs by BBR treatment, the expression of TLR4, TNF-α, IL-1β, and IL-6 were markedly decreased, and the nuclear translocation of NF-κB were blocked. Conclusion: BBR can reduce systemic low-grade inflammation of T2DM mice to alleviate disease, this effect may be through regulating the gut microbes or inhibiting TLR4 signaling pathway.

Keywords: Berberine, type 2 diabetes, chronic inflammation, intestinal microbes, TLR4

Introduction

Type 2 diabetes mellitus (T2DM) is a common endocrine disease that is increasing markedly in the general population worldwide, which has become a major public health challenge [1]. The pathogenesis of T2DM is multifactorial. The genetic, immune dysfunction, systemic low-grade inflammation, obesity, lack of activity, high-fat diet, neuropsychiatric and other relevant factors involve in development of T2DM. Among of them, the systemic low-grade inflammation is a vital pathogenic mechanism [1, 2]. Currently, many studies showed that lipopolysaccharide (LPS) was an important triggering factor to the inflammation. High-fat diet (HFD) could induce the numbers change of major gut microbes that lead to serum LPS and free fatty acid (FFA) levels rising, liver triglycerides aggregating, following trigger systemic low-grade inflammation that cause the occurrence of diabetes and hepatic insulin resistance [3]. LPS could combine with TLR4-CD14 complex on surface of the innate immune cell to induce systemic low-grade inflammation, thereby leading to systemic insulin resistance. However, TLR4-deficient mice were protected from HFD induced insulin resistance [4]. These researches suggested that LPS might activate TLR4-NF-κB signal pathway causing chronic subclinical inflammation and promoting the occurrence of insulin resistance.

It has been proved that gut microbes play an important role in the occurrence and development of T2DM. Burcelin R et al found that the

numbers of gut microbes were significantly changed in T2DM patients [5]. Currently, it has been confirmed that normal adults carry 1~2 kg intestinal microbes containing about 1000 different species and 10^{14} bacteria, which is almost 10 times as many as the self cells of body and these bacteria encoding genes are more than 100 times those of self genes [6]. These intestinal bacteria can utilize nutrients sufficiently and generate metabolites to affect the body's energy metabolism phenotype that is associated with the susceptibilities of obesity, insulin resistance, metabolic syndrome, fatty liver disease, diabetes and other metabolic diseases [7]. These results suggested that intestinal flora involved in development of T2DM, therefore, regulation gut microbes may be a beneficial way to prevent and control diabetes.

Berberine (BBR) is a main ingredient of traditional Chinese medicine derived from root and bark of Ranunculaceae Coptis plant [8]. As a broad-spectrum antibiotic, BBR can resist or kill a variety of bacteria, fungi, viruses, protozoa, and worms, so that it is widely used to treat gastroenteritis, bacterial dysentery and other infectious diseases in the clinic. In recent years, some researchers found that BBR could relieve T2DM and insulin resistance by decrease glucose uptake, increase of glucose consumption, sensitivity and secretion of insulin, regulation lipid metabolism [9, 10]. However, the bioavailability and plasma concentration of BBR are very low in T2DM patients when oral administration, the phenomenon can't explain the mechanism of hypoglycemic and hypolipidemic effects of BBR [11]. Thus, we speculate that the mechanism of BBR treatment T2DM may be to decrease systemic low-grade inflammation via regulating gut microbiota and reducing the levels of LPS in serum and inhibiting the activation of LPS-TLR4-NF-κB signaling pathway.

In this paper, we explored the hypoglycemic mechanism of BBR through construction mice model of T2DM and administration of BBR. The results showed that BBR could reduce systemic low-grade inflammation of T2DM mice to relieve disease, this effect may be through regulating the gut microbes or inhibiting TLR4 signaling pathway.

Materials and methods

Animals

Male Kunming mice, weighing 20-22 g, were purchased from the Experimental Animal Center in South of China University, who were allowed to eat a standard diet and were accommodated for one weeks to the experimental condition of 20 ± 2°C, 60 ± 5% humidity [12]. The study was conducted in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals from the Association for the Committee for Animal Experiments of the National Centre.

Mice were randomly divided into 4 groups, named as Control (n = 10), T2DM models (n = 10), T2DM plus BBR oral gavage (T2DM+BBR ig, n = 10) and T2DM plus BBR intraperitoneal injection (T2DM+BBR ip, n = 10). T2DM mice were induced by low-dose streptozotocin (STZ, Sigma, Chemicals, St. Louis, MO, USA) treatment (30 mg/kg, ip) and HFD (70% standard diet, 12% lard, 9% yolk powder, 9% plantation white sugar). The animals with fasting plasma glucose (FPG) over 11.1 mmol/L after injected STZ for one week were diagnosed with diabetes [13]. T2DM model mice were treated by BBRig (100 mg/kg/d) or BBR ip (10 mg/kg/d) for consecutively 6 weeks [14]. After fasting for 12 h, mice were sacrificed to harvest serum, pancreas and colon etc standby application [15].

The FPG and fasting insulin (FIns)

After T2DM mice model established successfully, FPG of mice were biweekly measured with a drop of tail vein blood by standard Glucometer (Sinocare Inc., Changsha, China) after fasting 12 hours. Fins level in serum of every group mice after BBR treatment six weeks were assayed according to the manufacturer's instructions of ELISAkit (Mouse Insulin ELISA kit, Beijing Dong Songs Biological Technology, Beijing, China). Meanwhile, homeostasis model assessment of insulin resistance (HOMA-IR) were assessed for all animals, HOMA-IR = [FINS (mU/L) × FPG (mmol/L)]/22.5 [16].

Intestinal microbes

0.1 g fresh excrement of mouse was taken from its colon and placed in sterile centrifuge tube containing 0.9 mL anaerobic dilution buffer.

Table 1. Sequences of primers employed for qRT-qPCR amplifications

Human Gene	Primers (5'-3')	Production size (bp)	Mouse Gene	Primers (5'-3')	Production size (bp)
TLR4	AGTCCAGAAAAGGCTCCCAG	116	TLR4	CAGATAGTCGGAGGCTGTCAA	123
	AATCCCCTGAGGCATTTAGGC			CTGGGAAGGGGGATAACATT	
TNF-α	CAGGGCAATGATCCCAAAGT	65	TNF-α	TCTGTCCCCTCCACACTCTC	116
	TCAATCGGCCCGACTATCTC			GCAACCCTTATTCTCGCTCA	
IL-1β	TCTTTCAACACGCAGGACAG	133	IL-1β	GTGTGGTATTGGTGGGAAGG	120
	TCCAGGGACAGGATATGGAG			CTGCCTGTGGGTTCTTTTTC	
IL-6	AACAACAATCTGAGGTGCCCATGCTAC	150	IL-6	GACCCTCACTCCTTCCCTTC	121
	AAATGCCAGCCTGCTGACGAAC			CACTCTTCACACCCCTCTCC	
18s	GCACCACCACCACGGAATCG	120	GAPDH	GGTTGTCTCCTGCGATTTCA	183
	TTGACGGAAGGGCACCACCAG			TGGTCCAGGGTTTCTTACTCC	

The quantitative and qualitative tests of each sample were assayed by bacteria plate count method [17, 18]. The experimental steps are as follows: (1) cultivation and identification of gut bacteria with select medium. Above excremental sample of each mouse was serial dilute into 10^{-8} by 10 times dilution method. Then, 10^{-3}, 10^{-4} and 10^{-5} dilute solutions 50 μL were respectively taken to inoculate on five kinds of selective mediums that were TPY, LBS, BBE, PSE and EMB, which adapted to the growth of Bifidobacteria, Lactobacillus, Bacteroides, Enterococcus and Enterobacteriaceae respectively. The Bifidobacteria, Lactobacillus and Bacteroides were cultivated in anaerobic culture bag of TPY, BBE and LBS selective mediums for 48-72 h, whereas, Enterococcus and Enterobacteriaceae were cultured in a conventional incubator for 24-48 h. Finally, each bacterial genus were identified by gram staining and biochemical assay. (2) Bacterial count: selection plates of 30-300 colonies density, the average of bacterial colonies in same dilution of every group mice was calculated, then according to average bacterial colonies, bacterial numbers of per gram sample was counted by formula CFU/g (mL) = average colonies of a sample dilution × dilution ratio × 50 [19]. Finally, the results were adopted from common logarithm with the form of log10 CFU/g.

Hematoxylin-eosin (H&E) staining and immunohistochemistry

The pancreas tissues were embedded with paraffin and section slides after fixed 24 h in 10% buffered formalin (pH 7.2). The slides were stained by hematoxylin and eosin (H&E) for histopathological analyses.

Immunohistochemical staining (IHC) of pancreas tissue slides was performed. Immunohistochemical localization of macrophages was conducted using antibody against CD11b (Boster Biological Technology, Co. LTD, Wuhan, China). The sections were deparaffinized and blocked endogenous peroxidase. After nonspecific fixation was blocked with 10% goat serum in PBS, the sections were incubated with the primary antibodies (diluted 1:50) overnight at 4°C refrigerator. After further washing in PBS, the slides were processed using PV-9001 kit (Zhong Shan Jin Qiao Co., Beijing, China), and the peroxidase activity was assayed with diaminobenzidine (Sigma; St Louis, MO). Finally, the sections were counterstained with hematoxylin and mounted using conventional methods [20]. At last, the slides were observed under a microscope.

Cells culture

THP-1 cells were purchased from Shanghai Cell Resource Center Institute of Life Science that cultured in RPMI-1640 medium that contains 10% fetal bovine serum. Cells were inoculated in 6-well plates and stimulated 24 h with 160 nM phorbol 12-myristste 13-acetate (PMA, Sigma, Chemicals, St. Louis, MO, USA) for inducing them differentiation to MΦs [21]. MΦ were divided into four groups (a. Control; b. LPS+PBS; c. LPS+BBR 5 μM; d. LPS+BBR 10 μM) and stimulated up to 24 h by LPS (100 ng/mL) and BBR for standby application.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNAs of frozen pancreas tissues and MΦs treated by BBR were extracted by Trizol reagent

Table 2. The levels of fasting plasma glucose in mice

Week	Groups			
	Control (mmol/L)	T2DM models (mmol/L)	T2DM+BBR ig (mmol/L)	T2DM+BBR ip (mmol/L)
0	4.82±1.32	12.34±1.35#	12.27±2.31	11.87±2.86
2	4.35±1.07	11.37±2.17#	8.86±1.17&	6.35±1.34&
4	4.33±0.87	10.68±1.47#	6.33±1.57&,*	4.87±1.21&,*
6	4.62±1.13	10.67±1.21#	5.47±0.21&,*	4.63±0.67&,*

Note: #, P<0.05 compared with control in the same weeks; *, P<0.05 compared with T2DMmodels in the same weeks; &, P<0.05 compared with 0 weeks in the same group.

Table 3. The levels of FIns and HOMA-IR in mice

Group	FIns (uIU/ml)	HOMA-IR
Control	34.45±3.81	5.07±0.31
T2DM models	55.91±3.38#	6.39±0.43#
T2DM+BBR ig	40.83±5.19*	5.41±0.32*
T2DM+BBR ip	38.68±7.37*	5.19±0.47*,&

Note: FIns, Fasting insulin level; HOMA-IR, homeostasis model assessment estimated insulinresistance index. #, P<0.05 compared with control; *, P<0.05 compared with T2DM models; &, P<0.05 compared with T2DM+BBR ig.

(Invitrogen, Carlsbad, CA). These RNAs concentration were detected by spectrophotometer. Reverse transcription was performed with a cDNA synthesis kit (Invitrogen, Carlsbad, CA). A SYBR Green mix kit (Tiangen Biotechnology CO., Beijing, China) was applied in the PCR according to the manufacturer's instructions [22]. Data were presented as the ratio of genes to 18S or GAPDH mRNA and assessed with GraphPad Prism 5 software. Gene sequences of primers and the amplification product sizes are shown in **Table 1**.

Western-blot

Proteins of MΦs treated by BBR were extracted by cells nuclear and cytoplasmic protein extraction kit (Beyotime Biotechnology CO., Changsha, China). The protein concentrations were determined by BCA kit. These proteins were separated by SDS polyacrylamide gelelectrophoresis, transferred to a nitrocellulose membrane and blocked with 5% non-fat milk in TBST buffer. The membranes were respectively incubated with primary antibodies against TLR4 and NF-κB p65 (Bioworld Technology, Inc., minnesota, USA) overnight at 4°C. After washing three times with TBST, the membranes were incubated with the secondary antibody (Bioworld Technology, Inc., minnesota, USA) for 1 h at room temperature. Then these membranes were washed three times. An enhanced chemiluminescence detection kit was applied to visualize specific bands. QuantityOne software was employed to detect optical densities [23].

Statistical analysis

The results were expressed as mean ± SEM. Experimental data were statistically analyzed using One-way ANOVA test by SPSS18.0, when P<0.05 as statistically significant difference.

Results

The FPG was reduced after administrated BBR in T2DM mice

The results showed that the FPG increased significantly in T2DM mice compared with the normal control group (P<0.05). To test whether BBR can reduce FPG, T2DM mice were treated with BBRig (100 mg/Kg/d) or BBR ip (10 mg/Kg/d), the results indicated that the FPG decreased significantly in the groups of T2DM+BBR ig and T2DM+BBR ip (P<0.05), but the FPG was declined to normal level at the end of fourth week in the group of T2DM+BBR ip and at the end of sixth week in the group of T2DM+BBR ig (FPG<7.0 mmol/L) (**Table 2**). These results suggested that BBR could reduce FPG of T2DM mice, and the effect of BBR ip was significantly faster than that of BBR ig (P<0.05).

The FIns and HOMA-IR of T2DM mice were reduced after treatment with BBR

To test whether BBR can reduce FIns and HOMA-IR, the FIns of mice were detected after treatment with BBR, and estimated HOMA-IR by the homeostasis model assessment. As indicated that the FIns and HOMA-IR of T2DM mice increased significantly compared with normal control, while these parameters decreased markedly in the groups of T2DM+BBR ig and T2DM+BBR ip (P<0.05). There were no difference of FIns between T2DM+BBR ig and T2DM+BBR ip groups (P>0.05), but the HOMA-IR of T2DM+BBR ig group was higher than that of in T2DM+BBR ip group (P<0.05) (**Table 3**).

Table 4. The numbers of intestinal microflora in every group of mice ($\bar{x}$±s, Log10 CFU/g muck)

Groups	Enterobacter	Enterococcus	Lactobacillus	Bifidobacterium	Bacteroidetes
Control	8.13±0.17	6.57±0.23	7.34±0.15	8.90±0.23	8.38±0.14
T2DM models	9.01±0.05$^{\#}$	7.87±0.14$^{\#}$	5.59±0.32$^{\#}$	5.47±0.31$^{\#}$	6.63±0.41$^{\#}$
T2DM+BBR ig	7.97±0.12$^{\$}$	6.36±0.21$^{\bullet}$	7.28±0.12$^{\bullet}$	7.83±0.27$^{\bullet}$	7.88±0.27$^{\bullet}$
T2DM+BBR ip	8.32±0.17$^{\$,\star}$	7.18±0.16$^{\$,\&}$	5.73±0.28$^{\$,\&}$	6.34±0.14$^{\$,\&}$	6.96±0.35$^{\$,\&}$

Note: $^{\#}$, P<0.01 compared with control; $^{\$}$, P<0.05 compared with T2DM models; $^{\bullet}$, P<0.01compared with T2DM model; $^{\&}$, P<0.01 compared with T2DM+BBR ig; *, P<0.05 compared withT2DM+BBR ig.

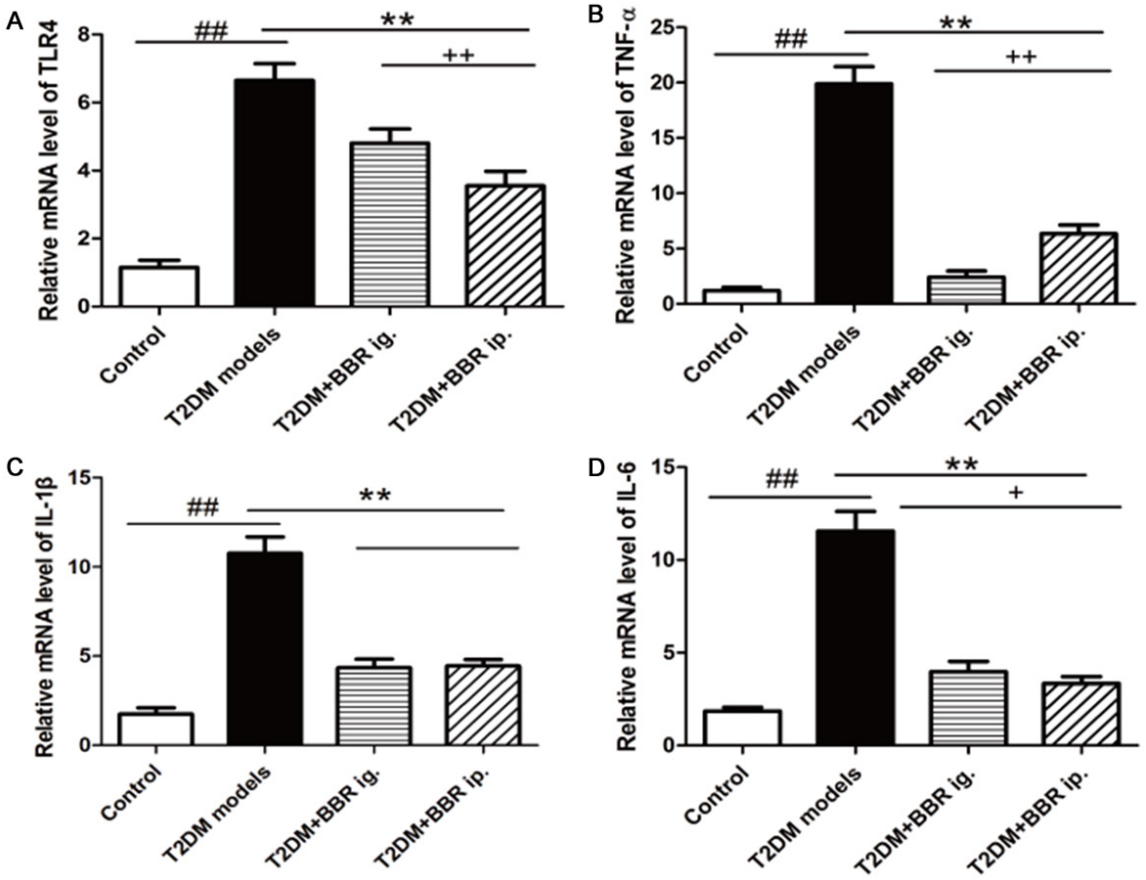

Figure 1. qRT-PCR was performed to analysis mRNA levels of TLR4 and TNF-α, IL-1β, IL-6 in pancreas of T2DM mice after BBR treatment. ##, P<0.01 compared with control; **, P<0.01 compared with T2DM models; ++, P<0.01 compared with T2DM+BBR ig; +, P<0.05 compared with T2DM+BBR ig.

The intestinal microflora of T2DM mice were adjusted by BBR

To further verify whether BBR can change intestinal microflora, the main microbes were detected after the T2DM mice administration of BBR for six weeks. The results showed that numbers of *Enterobacter*, *Enterococcus* increased markedly, while the numbers of *Lactobacillus, Bifidobacterium, Bacteroides* decreased significantly in T2DM mice when compared with normal control (P<0.05). However, amounts of *Enterobacter* and *Enterococcus* decreased significantly and amounts of *Lactobacillus*, *Bifidobacterium* and *Bacteroidetes* increased significantly in the groups of T2DM+BBR ig and T2DM+BBR ip when compared with T2DM mice (P<0.05) (**Table 4**). Furthermore, numbers of *Enterobacter* and *Enterococcus* in the group of T2DM+BBR ig were significantly lower than those of T2DM+BBR ip group, meanwhile, numbers of *Lactobacillus*, *Bifidobacterium* and

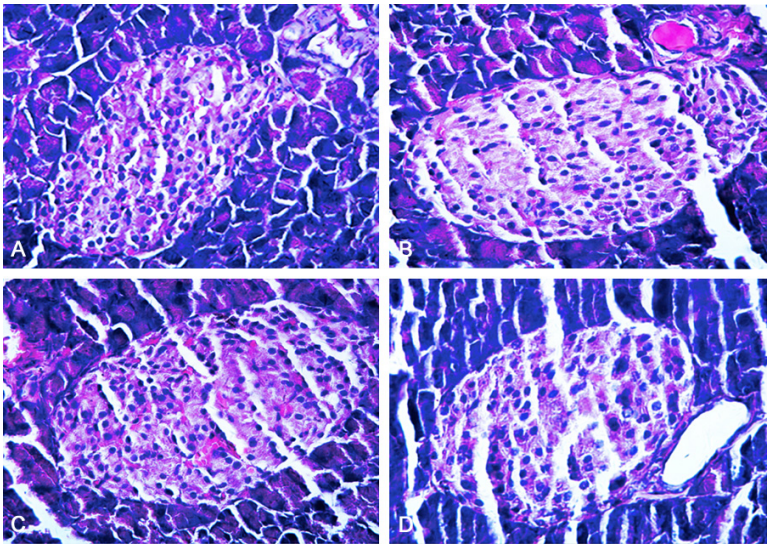

Figure 2. Observed the pathological changes in pancreatic islets of mice by H&E staining. A. Control group; B. T2DM models; C. T2DM+BBR ig; D. T2DM+BBR ip. H&E staining 400 ×.

Bacteroidetes were markedly higher than those of T2DM+BBR ip group (P<0.05) (**Table 4**).

The mRNA levels of TLR4 and TNF-α, IL-1β, IL-6 decreased in pancreatic tissues of T2DM mice after administration of BBR

To verify the role of BBR in regulation of TLR4 and inflammation factors, qRT-PCR was performed to detect the mRNA levels of TLR4 and TNF-α, IL-1β, IL-6 in pancreatic tissues of T2DM mice. The results showed that the mRNA levels of TLR4 and TNF-α, IL-1β, IL-6 markedly increased in T2DM models compared with normal control (P<0.05). However, these parameters were decreased significantly in the groups of T2DM+BBR ig and T2DM+BBR ip (P<0.01) (**Figure 1**). Furthermore, in the group of T2DM+BBR ig, the mRNA levels of TLR4 and IL-6 were significantly higher than those of T2DM+BBR ip group (P<0.01) (**Figure 1A**, **1D**). The mRNA level of TNF-α in the group of T2DM+BBR ig was lower than those of T2DM+BBR ip group (P<0.05) (**Figure 1B**). There were no differences of mRNA level of IL-1β between T2DM+BBR ig and T2DM+BBR ip groups (P>0.05) (**Figure 1C**).

Pathological damage and MΦ infiltration were decreased in pancreatic islets of T2DM mice after administrated BBR

H&E staining and IHC were performed to examine whether BBR can decrease pathological damage and MΦ infiltration in pancreas of T2DM mice. H&E staining showed that cells arranged neatly, cytoplasm plump and structure clearly in the pancreas islet of normal control mice (**Figure 2A**). Whereas, the number of cells was reduced, and the cell volume increased, shape was irregular and different of nuclear size in the pancreatic islet of T2DM mice. Meanwhile, a few of cells were vacuolated, arrangement disordered and nuclear pyknotic (**Figure 2B**). In the groups of T2DM+BBR ig and T2DM+BBR ip, pancreatic islet cells increased significantly, distributed uniform, equal of nuclear size without nuclear pyknotic compared with T2DM mice (**Figure 2C**). There were no pathological difference between T2DM+BBR ig and T2DM+BBR ip groups (**Figure 2C**, **2D**).

IHC revealed that MΦs were positive cells of immune response that represent dark brown in pancreas islets. In normal control group, there was no MΦs infiltration in the pancreas islet (**Figure 3A**). However, MΦs infiltration was increased markedly in pancreas islets of T2DM models (**Figure 3B**). In the groups of T2DM+BBR ig and T2DM+BBR ip, MΦs infiltration was decreased significantly in pancreas islets. There were no difference of MΦs infiltration between T2DM+BBR ig and T2DM+BBR ip groups (**Figure 3C**, **3D**).

The effect of BBR to the expression of TLR4 and nuclear translocation of NF-κBp65 in THP-1 derived MΦ

The protein levels of TLR4 and NF-κBp65 were detected by western blot in THP-1 derived MΦs. The results showed that the protein levels of TLR4 of cytosol and NF-κBp65 of nuclear increased significantly in the group of LPS+PBS compared with normal control (P<0.05), while the above indexes decreased markedly in the groups of LPS+BBR (5 μM) and LPS+BBR (10 μM) (P<0.05) (**Figure 4A**, **4B**). Furthermore, these protein levels in the group of LPS+BBR (10 μM) were lower than those of LPS+BBR (5 μM) group (P<0.05) (**Figure 4**). Moreover, the

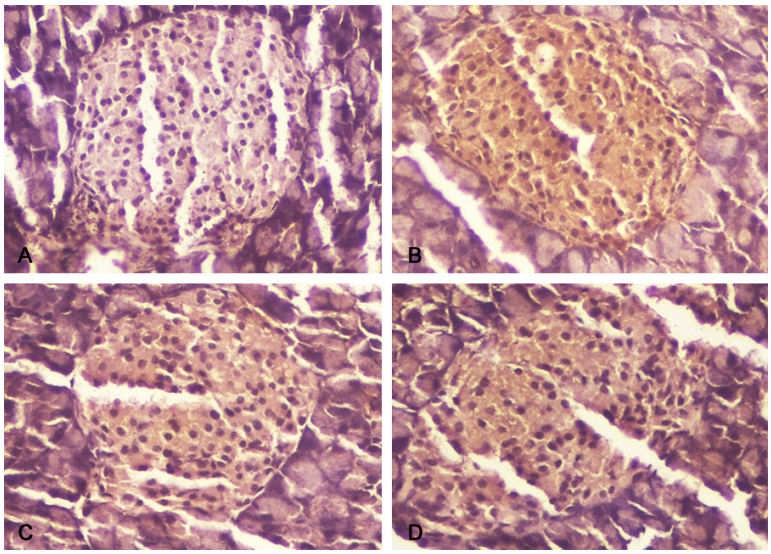

Figure 3. Observed MΦ infiltration in pancreas islets of mice by IHC. The MΦ is positive cells of immune response which represent dark brown. A. Control group; B. T2DM models; C. T2DM+BBR ig; D. T2DM+BBR ip. IHC 400 ×.

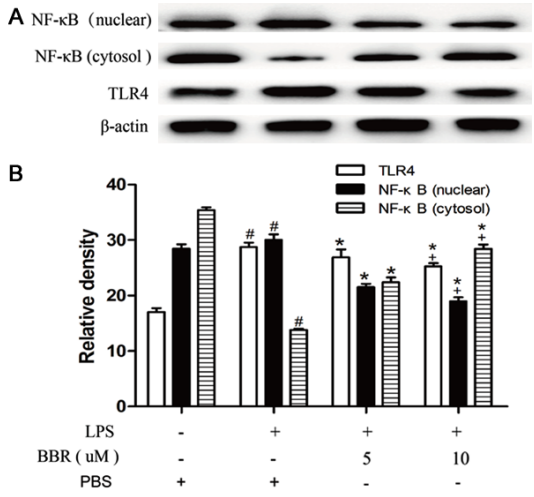

Figure 4. TLR4 protein level and nuclear translocation of NF-κBp65 were detected in MΦ by western blot after BBR treatment. A: Western blot detected the protein levels. B: The density value were analyzed by Quantity One software. #, P<0.05 compared with normal control; *, P<0.05 compared with LPS+PBS; +, P<0.05 compared with LPS+BBR (5 μM).

protein level of NF-κBp65 of cytosol decreased significantly in the group of LPS+PBS compared with normal control, and increased significantly in the groups of LPS+BBR (5 μM) and LPS+BBR (10 μM) compared with LPS+PBS (P<0.05). However, the protein level of NF-κBp65 of cytosol in the group of LPS+BBR (10 μM) was higher than that of LPS+BBR (5 μM) group (P<0.05) (**Figure 4A**, **4B**).

The mRNA levels of TLR4 and TNF-α, IL-1β, IL-6 decreased in THP-1 derived MΦ after BBR treatment

The mRNA levels of TLR4 and TNF-α, IL-1β, IL-6 in THP-1 derived MΦ were detected by qRT-PCR. The results showed that the mRNA levels of TLR4 and TNF-α, IL-1β, IL-6 increased significantly in the group of LPS+PBS compared with normal control, and decreased significantly in the groups of LPS+BBR (5 μM) and LPS+BBR (10 μM) compared with LPS+PBS (P<0.01) (**Figure 5**). Moreover, these parameters in the group of LPS+BBR (10 μM) were lower than those of LPS+BBR (5 μM) (P<0.05) (**Figure 5C**, **5D**). These results suggested that BBR could decrease the mRNA levels of TLR4 and TNF-α, IL-1β, IL-6 in a dose-effect manner.

Discussion

In recent years, with the deepening research on diabetes, many researchers have found that intestinal microbes imbalance was an important factor of systemic low-grad inflammation that associated with the occurrence of T2DM [24, 25]. Many previous reports indicated that BBR was rarely absorbed in intestinal tract so that the hypoglycemic mechanism is unclear in T2DM patients, we speculated that BBR might plays a role through regulating intestinal microbes. In order to investigate the pharmacological mechanism of BBR, we further established mice model of T2DM and administra-tion of BBR. The results suggested that BBR could participate in regulating intestinal microbes that promote proliferation of *Bifidobacterium*, *Lactobacillus* and *Bacteroides* and inhibit reproduction of *Enterobacter* and *Enterococcus*. Meanwhile, the effect of BBR ip on regulating gut microbes was lower than that of BBR ig, it was suggested that BBR ip plays its biologic role through blood circulation, while BBR ig plays a direct role in intestinal microbes. The gram-negative bacterium of intestinal tract could produce abundant LPS to induce systemic low-grad inflammation. Shan *et al* found that the levels of LPS, FPG, FIns and IR increased significantly in T2DM rats, but these parame-

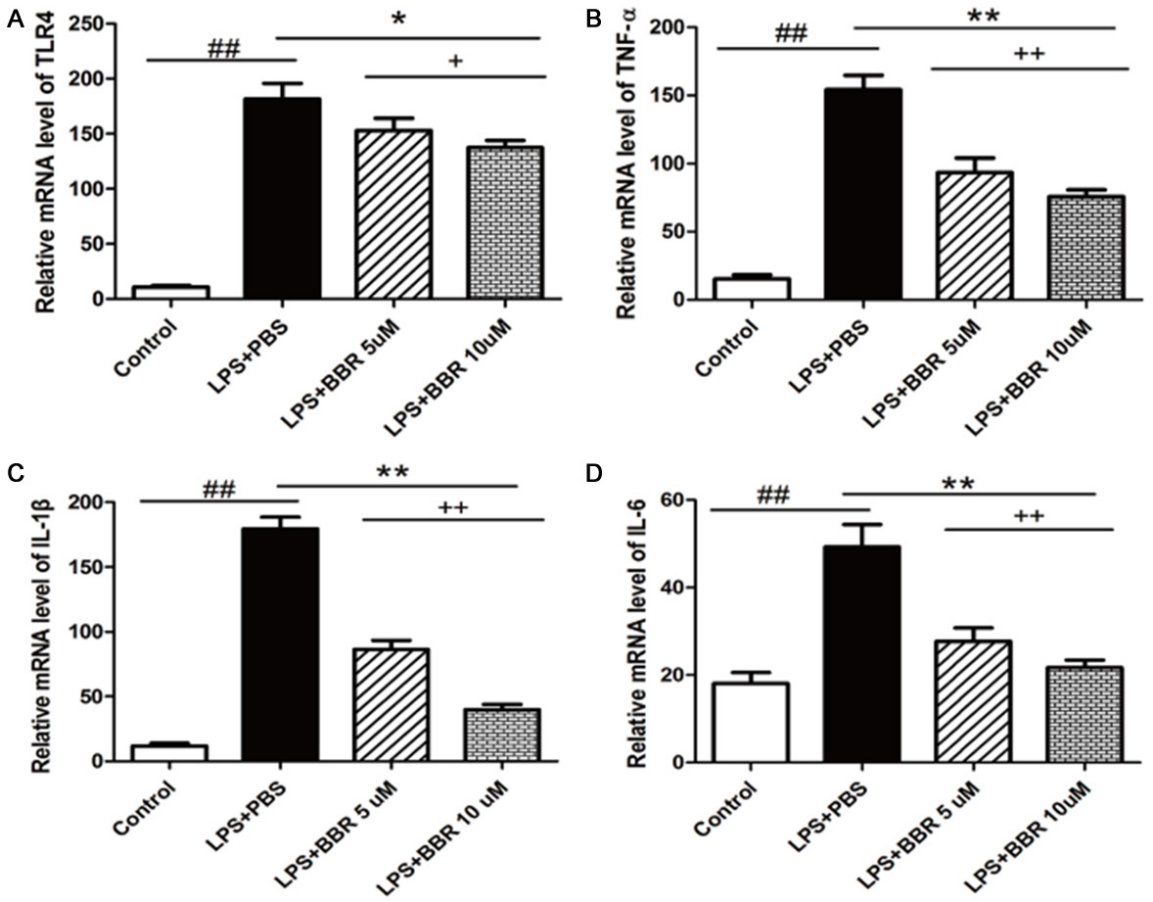

Figure 5. qRT-PCR was performed to detect mRNA levels of TLR4 and TNF-α, IL-1β, IL-6 in MΦ after administrated BBR. ##, P<0.01 compared with normal control. **, P<0.01 compared with LPS+PBS. ++, P<0.01 compared with LPS+BBR (5 μM). +, P<0.05 compared with LPS+BBR (5 μM).

ters decreased markedly after treatment with BBR ig [26]. The results suggested that BBR could relieve diabetes by reducing serum LPS level which was consistent with our study results. In addition, our study showed that the level of FPG was decreased in T2DM mice after administration of BBR ig or BBR ip for 6 weeks, its mechanism may be related to regulating gut microbes and further reducing serum LPS level. Moreover, the role of hypoglycemicin group of T2DM+BBR ip was better than the group of T2DM+BBR ig, which might be associated with the BBR ip directly inhibit systemic low-grad inflammation of mice to improve the insulin sensitivity.

LPS could combine with TLR4 on the surface of innate immune cells to trigger the translocation of NF-κB, further induce the expression of TNF-α, IL-1β and IL-6 and promote the occurrence of systemic chronic inflammation of self body [4]. The systemic chronic inflammation might cause damage of the pancreas islet cells and insulin resistance to induce type 2 diabetes [27]. In this study, our data showed that BBR could inhibit the expression of TLR4, TNF-α, IL-1β and IL-6 to reduce systemic chronic inflammation, thereby decreased insulin resistance and pancreas islet cells damage to relieve diabetes. The mRNA level of TLR4 in T2DM+BBR ig group was higher than that of T2DM+BBR ip group, because BBR ig might directly act on intestinal microbes to reduce the generation of LPS, thereby inhibit the activated of LPS-TLR4 axis to decrease the release of inflammatory cytokines. Furthermore, the studies *in vitro* demonstrated that BBR could decrease the expression of TLR4 and block nuclear translocation of NF-κB, reduce the expression of TNF-α, IL-1β and IL-6, which further suggested that BBR might play a role by directly inhibit TLR4-NF-κB signaling pathways.

MΦ could play an important role in the process of development and occurrence of T2DM. Meshkani et al found that MΦ could immerse in different tissues to release inflammatory cytokines and chemokines that cause systemic chronic inflammation, lead to islet β cell dysfunction and insulin resistance, thereby promote the occurrence of diabetes [28]. Furthermore, our findings suggested that pathological damage and MΦs infiltration increased significantly in pancreatic islets of T2DM mice, but the pathological characteristics were alleviated markedly after treatment with BBR. These data suggested that BBR could reduce the degrees of MΦs infiltration and pathological damage in pancreatic islets of T2DM mice, thereby decreased FPG and HOMA-IR to alleviate diabetes. Further work needs to be done to determine whether BBR could decrease the LPS level in serum of T2DM mice and degree of MΦs infiltration in peripheral tissues to reduce the release of inflammatory cytokines and alleviate diabetes.

Conclusions

Here, we demonstrated that BBR could suppress systemic low-grad inflammation to alleviate type 2 diabetes, this effect may be through adjusting gut microbes and inhibiting TLR4-NF-κB signaling pathway.

Acknowledgements

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jianhua Xiao, Institute of Pathogenic Biology, Medical College, University of South China, Hengyang 421001, China. E-mail: jhxiao223@163.com

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