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The phosphodiesterase type 4 inhibitor roflumilast suppresses inflammation to improve diabetic bladder dysfunction rats

Honglin Ding[1,2] · Peng Zhang[3] · Ning Li[1] · Yili Liu[1] · Ping Wang[1]

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Abstract

Purpose To demonstrate that phosphodiesterase type 4 (PDE4) inhibitors could potentially treat diabetic bladder dysfunction (DBD) through modulation of the systemic inflammatory response.

Methods In this 6-week study, 60 female Sprague–Dawley rats were divided into three groups: (i) vehicle-treated control rats; (ii) vehicle-treated streptozocin (STZ)-injected rats; and (iii) roflumilast-treated STZ-injected rats. Oral roflumilast (5 mg/kg/day) was administered during the last 4 weeks of STZ injection to induce diabetes in the test group. At 6 weeks, a urodynamic study was performed in each group. The expression of nuclear factor kappa B (NF-κB), tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, and IL-1β in detrusor smooth muscle (DSM) was analyzed using quantitative reverse transcription-polymerase chain reaction and Western blotting.

Results A significant decrease in bodyweight and significant increases in bladder weight and blood glucose level were observed in the diabetic rats and were not ameliorated by roflumilast treatment. Cystometry showed the increased bladder capacity, voiding volume, residual urine volume, and voiding interval in the diabetic rats and the prevention of these changes by roflumilast. These changes were accompanied by significantly enhanced expression of NF-κB, TNF-α, IL-6, and IL-1β in DSM tissue from diabetic rats. Furthermore, roflumilast attenuated the expression of inflammatory factors in DSM tissue.

Conclusions Oral treatment with roflumilast in diabetic rats improves bladder function and inhibits the expression of inflammatory factors in DSM tissue, indicating that PDE4 is a potential therapeutic target for DBD.

Keywords Phosphodiesterase type 4 · Diabetic bladder dysfunction · Diabetes · Streptozocin · Inflammatory

Introduction

In recent years, the improvement in living standards and the growth in the number of elderly people have led to a higher incidence of diabetes. Diabetes mellitus (DM) is a metabolic disease whose main manifestation is chronic hyperglycemia [1, 2]. Diabetes is a common disease and a serious threat to public health. Its incidence has increased year over year, and approximately 80% of diabetic patients have lower urinary tract symptoms (LUTS) [1, 3]. For diabetes, the most common type of urologic disorder is diabetic bladder dysfunction (DBD), which is a group of clinical syndromes with the main clinical manifestations of bladder sensory decline, increased bladder residual urine volume, and decreased detrusor smooth muscle (DSM) contractility [4, 5]. In asymptomatic patients with diabetes, increased bladder volume at first sensation to void and decreased DSM contractility were found with a resultant increase in the post-void residual urine volume [6]. The mechanisms underlying the diabetes-induced symptoms of DBD involve the nerves, DSM, bladder mucosa and other factors [4]. As a result, DBD has become a common issue that people with diabetes experience.

Traditionally, the first-line treatment for DBD is behavioral therapy that may be combined with pharmacological management, followed by oral antimuscarinic treatment. However, the side effects and intolerance to antimuscarinic drugs limit their clinical utility [7, 8]. Anticholinergic drugs

✉ Ning Li
air-nick@hotmail.com

1 Department of Urology, Fourth Affiliated Hospital, China Medical University, 4 Chongshan East Road, Shenyang, Liaoning, China

2 Department of Urology, Affiliated Hospital, Chifeng University, 42 Wangfu Street, Chifeng, Neimeng, China

3 Department of General Surgery, Shenyang 242 Hospital, 3 Leshan Road, Shenyang, Liaoning, China

are not sufficient for the treatment of DBD because diabetes-induced oxidative stress causes DSM changes. This stress is mainly due to the general inflammatory reaction induced by diabetes, and the effects on DSM contractility increase with the activation of inflammatory factors [4]. Generally, insulin resistance is closely related to a chronic low-level inflammatory state due to the continuous activation of various inflammatory mediators, including nuclear factor kappa B (NF-κB), tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, and IL-1β [9–11]. The inhibition of the inflammatory response can significantly improve the bladder dysfunction associated with type 2 diabetes or high-fat diet-induced obesity [12–14]. There is therefore a need for a drug that is directed against inflammatory reactions to treat DBD.

Phosphodiesterase type 4 (PDE4) plays a key role in the degradation of cyclic adenosine monophosphate (cAMP) in inflammatory cells as well as in vascular endothelial cells, smooth muscle cells, and related inflammatory keratinocytes [15]. PDE4 inhibitors can improve cAMP levels in airway smooth muscle to relieve the inflammation in the treatment of chronic obstructive pulmonary disease (COPD) [16]. In our previous experiments, roflumilast, a selective PDE4 inhibitor, was confirmed to attenuate inflammatory response in DSM and to relieve bladder dysfunction in a rat model of obesity [14]. In this work, a DBD model was established using streptozocin (STZ) to evaluate the interventional effects of the PDE4 inhibitor roflumilast on DBD through Western blotting and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analyses.

Materials and methods

Animals

Sixty adult female Sprague–Dawley (SD) rats (average weight 244.7 ± 5.6 g; China Medical University, Shenyang, China) were used in this study. All experimental procedures were approved by the Institutional Animal Care and Use Committee of China Medical University.

Induction of diabetes and treatment groups

The study animals were housed three per cage on a 12-h light–dark cycle. 60 rats were randomly selected to receive a single intraperitoneal injection of 65 mg/kg STZ diluted in 0.1 M citrate buffer solution (pH 4.5), as previously described [4, 17]. Controls were treated identically except that a similar volume of buffer was injected instead of STZ. 3 Days after STZ treatment, a blood sample was obtained from the tail vein to estimate the blood glucose level. The blood glucose level of these rats was checked after STZ injection to confirm the presence of diabetes (blood glucose 300 mg/dl). After 2 weeks, the diabetic rats were randomly divided into two groups. At this point, the experimental rats had been divided into three groups: (1) Con + vehicle, control rats receiving vehicle for 4 weeks; (2) STZ + vehicle, STZ-injected rats receiving the vehicle for 4 weeks; and (3) STZ + roflumilast, STZ-injected rats receiving roflumilast, a selective PDE4 inhibitor, for 4 weeks. Roflumilast (5 mg/kg/day; MedChemExpress, USA) or vehicle (sterile water used as a solvent for roflumilast) was administered orally by gavage during the last 4 weeks of feeding. All rats were weighed at 6 weeks, and urodynamic studies were conducted in ten rats from each group. The remaining rats were used for qRT-PCR and Western blotting experiments. The animals were then sacrificed in a carbon dioxide tank prior to the collection of bladder specimens; the bladder mucosa was separated under a microscope, and DSM tissue was preserved in liquid nitrogen.

Cystometry

Cystometry was performed with anesthetized rats, and general anesthesia was induced with 5% isoflurane/O^2 gas inspiration using a facial mask as described previously [14, 18, 19]. A catheter was inserted into the bladder dome after surgically exposing the bladder and was connected to a physiological pressure transducer and an injection pump (Dantec Menuet, Denmark). Cystometry was performed by infusing warm saline (37–38 °C) into the bladder at a flow rate of 12 ml/h. Five voiding events were recorded for each rat to assess bladder capacity (the volume of saline infused to induce voiding), voiding volume (the volume of micturition), residual urine volume (the volume of the post-void residual urine), maximum voiding pressure (the maximum pressure during voiding), and voiding interval (the interval between voids). The bladders assessed by cystometry were not used in other experiments.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was isolated from rat DSM strips using RNA Isolater Extraction Reagent (Vazyme Biotech Co., Ltd, China). Reverse transcription of total RNA was performed using the HIScriptII One Step qRT-PCR SYBR Green Kit (Vazyme Biotech Co., Ltd, China) according to the manufacturer's instructions. PCR was then performed using the synthesized mRNA on an ABI PRISM7500 sequence detection system with SYBR Green PCR Master Mix. Real-time PCR was carried out to analyze the mRNA expression of TNF-α, IL-6, IL-1β, NF-κB, and GAPDH using specific primers (Table 1). The primer design is the same as that in our previous article [14]. The PCR conditions were 50 °C for 5 min, followed by 95 °C for 30 s and then 58 °C for 40 s for a total of 40 cycles.

Table 1 Primers used for the qRT-PCR experiments

Gene	Forward	Reverse	Size (bp)
NF-κB	GCAAACCTGGGAATACTTCATGTGACTAAG	ATAGGCAAGGTCAGAATGCACCAGAAGTCC	123
IL-1β	TGACCCATGTGAGCTGAAAG	GGGATTTTGTCGTTGCTTGT	108
TNF-α	TACTGAACTTCGGGGTGATTGGTCC	CAGCCTTGTCCCTTGAAGAGAACC	295
IL-6	CAAGAGACTTCCAGCCAGTTGC	TTGCCGAGTAGACCTCATAGTGACC	614
GAPDH	GTTACCAGGGCTGCCTTCTC	ACCAGCTTCCCATTCTCAGC	152

bp base pairs

All reactions were run thrice and were normalized to GAPDH. Melt curves were utilized to analyze and assess the accuracy of the PCR results. The gene expression was evaluated by $2^{-\Delta\Delta Ct}$ method. The relative mRNA expression of each target gene was normalized to that of GAPDH.

Western blotting

Briefly, protein from the rat DSM strips was isolated and homogenized in a homogenizer with RIPA buffer (50 mM Tris, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, and 1% sodium deoxycholate). Protein concentrations were measured using the BCA Protein Assay Kit (Beyotime Biotechnology). Approximately 10–20 μg of the protein samples were separated in a denaturing 10% or 12% SDS-PAGE gel and transferred to a nitrocellulose membrane. The membranes were washed, blocked [5% bovine serum albumin (BSA) in Tris-buffered saline with Tween 20 (TBS-T)], and incubated with primary rabbit antibody against TNF-α (1:500; Abcam), IL-1β (1:1000; Abcam), NF-κB (1:1000; Abcam), or β-actin (1:2000; Cell Signaling Technology) or with mouse antibody against IL-6 (1:1000; Abcam) overnight at 4 °C. Secondary antibodies were conjugated to horseradish peroxidase. Visualization was performed with ECL Western blotting detection reagents (Beyotime Biotechnology). The density of the target protein relative to β-actin was evaluated using Gel-Pro32 software.

Statistical analysis

Data were further analyzed with GraphPad Prism 5.0 (GraphPad software, San Diego, CA, USA) and are expressed as the mean ± SEM (n = the number of strips or PCR samples and N = the number of rats). Statistical significance was assessed using one-way ANOVA, followed by Newman–Keuls comparison test; a P value of $P < 0.05$ was considered statistically significant.

Results

General characteristics

Two of the rats receiving STZ injection were excluded: one due to failure to induce diabetes and the other due to death. At 6 weeks, the body weight of the diabetic rats decreased significantly compared to that of the rats in the Con + vehicle group (STZ + vehicle 225.7 ± 15.8 g, $N = 19$; Con + vehicle 379.2 ± 13.1 g, $N = 20$; $P < 0.05$). The average blood glucose level of the diabetic rats was approximately four times higher than that of the control rats at 6 weeks (STZ + vehicle 451.8 ± 14.3 mg/dl, $N = 19$; Con + vehicle 105.4 ± 8.3 mg/dl, $N = 20$; $P < 0.05$). The bladder weights of the diabetic rats (198.2 ± 11.9 mg, $N = 19$) were approximately 1.7 times the weights of the control rats (112.7 ± 10.1 mg, $N = 20$; $P < 0.05$). However, oral roflumilast treatment did not cause a significant change in body weight (231.7 ± 13.9 g, $N = 19$; $P > 0.05$), bladder weight (192.7 ± 12.1 mg, $N = 19$; $P > 0.05$), or blood glucose (449.1 ± 13.9 mg/dl, $N = 19$; $P > 0.05$) relative to the corresponding parameters of the diabetic rats receiving vehicle.

Urodynamic changes in diabetic rats

During cystometry, the diabetic rats receiving vehicle exhibited increased bladder capacity (1.44 ± 0.1 ml, $N = 10$), voiding volume (1.24 ± 0.11 ml, $N = 10$) and residual urine volume (0.21 ± 0.05 ml, $N = 10$) compared to the corresponding parameters (0.63 ± 0.05 ml, 0.62 ± 0.06 ml, 0.02 ± 0.02 ml, $N = 10$; $P < 0.05$; Fig. 1a–d) of the normal rats. However, there was no significant difference in the maximum voiding pressure between the STZ + vehicle

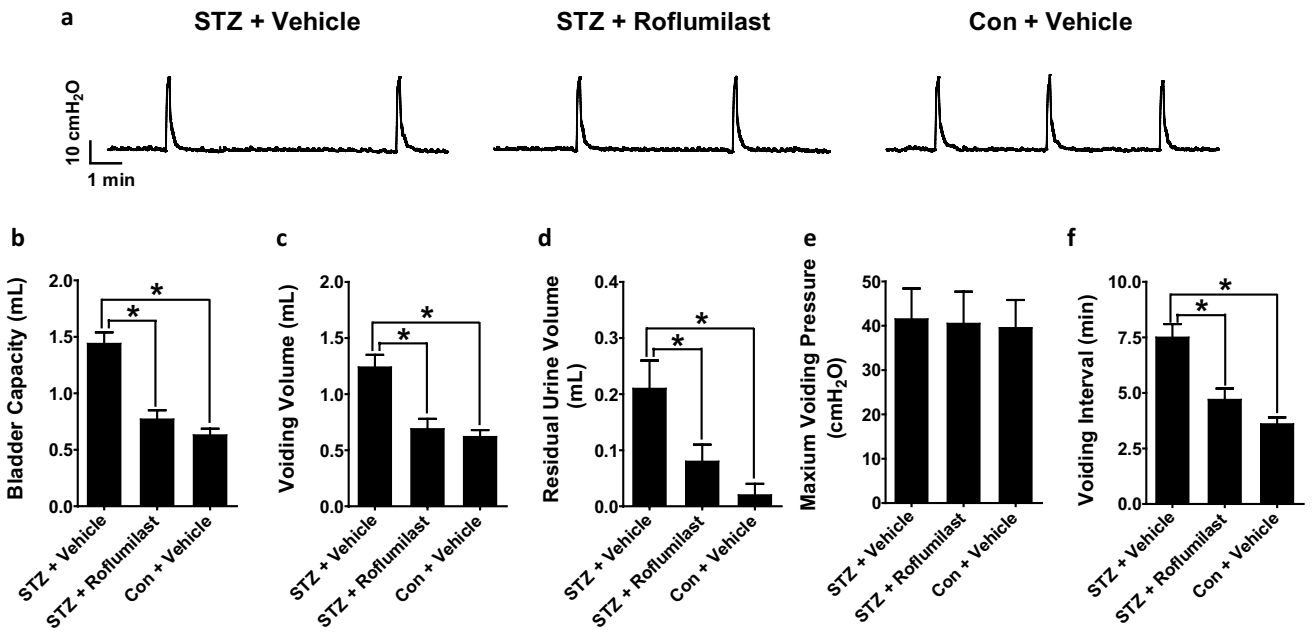

Fig. 1 Urodynamic changes in diabetic rats. **a** Representative cystometrogram illustrating the changes and effect of roflumilast on bladder function in diabetic rats. Increased bladder capacity (**b**), voiding volume (**c**), residual urine volume (**d**), and voiding interval (**f**) were observed in diabetic rats compared with those in normal animals ($N=10$ in each group; $^*P<0.05$ Con + vehicle versus STZ + vehicle). Furthermore, roflumilast improved the changed urodynamic parameters in diabetic rats ($N=10$ in each group; $^*P<0.05$ STZ + roflumilast versus STZ + vehicle). However, there was no significant difference in the maximum voiding pressure (**e**) among the three groups ($P>0.05$)

group (41.5 ± 6.9 cmH_2O, $N=10$) and the control group (39.5 ± 6.3 cmH_2O, $N=10$; $P>0.05$; Fig. 1a, e). Furthermore, there was a significant increase in the voiding interval (STZ + vehicle 7.5 ± 0.6 min, $N=10$; Con + vehicle 3.6 ± 0.3 min, $N=10$; $P<0.05$; Fig. 1a, f), indicating that DBD was already induced.

Therapeutic effect of PDE4 inhibitor on bladder dysfunction in diabetic rats

After 4 weeks of treatment with roflumilast, a selective PDE4 inhibitor, the bladder function parameters, bladder capacity (0.77 ± 0.08 ml, $N=10$), voiding volume (0.69 ± 0.09 ml, $N=10$), residual urine volume (0.08 ± 0.03 ml, $N=10$), and voiding interval (4.7 ± 0.5 min, $N=10$), were significantly improved in the rats in the diabetic group compared to those in the STZ + vehicle group ($P<0.05$; Fig. 1a–d, f). However, the maximum voiding pressure was still similar for the STZ + roflumilast rats (40.5 ± 7.2 cmH_2O, $N=10$; $P>0.05$; Fig. 1a, e). These data indicate that roflumilast, a selective PDE4 inhibitor, has specific therapeutic effects on DBD.

Hyperglycemia upregulated the inflammatory factor protein activity and mRNA level

Next, we examined the mRNA and protein expression levels of NF-κB, the upstream mediator of cytokine transcription that plays a pivotal role in regulating a variety of genes involved in the inflammatory response, in bladder tissue. We observed that the mRNA level of NF-κB (0.79 ± 0.05, $n=16$, $N=7$) in DSM tissue from the STZ + vehicle group was significantly higher than that of the Con + vehicle group (0.45 ± 0.03, $n=16$, $N=7$; $P<0.05$; Fig. 2a). With the increase in the mRNA level of NF-κB, the mRNA level of other inflammatory factors, such as TNF-α (0.71 ± 0.06, $n=16$, $N=7$), IL-6 (0.69 ± 0.06, $n=16$, $N=7$), and IL-1β (0.65 ± 0.05, $n=16$, $N=7$), increased in the DSM of diabetic rats, and these levels were significantly different from those in the DSM of the control rats (0.35 ± 0.04; 0.31 ± 0.04; 0.28 ± 0.03, $n=16$, $N=7$; $P<0.05$; Fig. 2a–d).

To confirm the results of the inflammatory factor changes caused by diabetes, the protein expression levels were evaluated in DSM tissue. The results demonstrated that

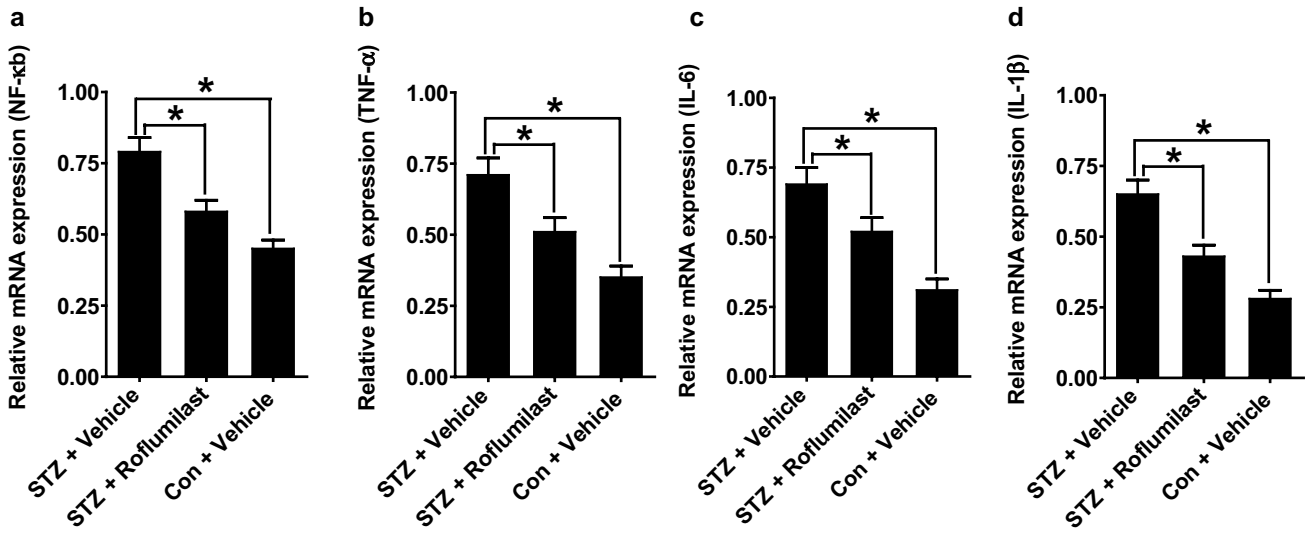

Fig. 2 Oral roflumilast treatment attenuated the enhanced mRNA expression of inflammatory factor in diabetic rat DSM. qRT-PCR analyses indicated enhanced mRNA expression of NF-κB (**a**), TNF-α (**b**), IL-6 (**c**), and IL-1β (**d**) in vehicle-treated STZ-injected rats compared with those in normal rats ($n=16$, $N=7$ for each group; $^*P<0.05$ Con + vehicle versus STZ + vehicle). The group of roflumilast-treated STZ-injected rats showed significantly decreased mRNA expression in the DSM compared with the expression in the DSM of vehicle-treated STZ-injected rats ($n=16$, $N=7$ for each group; $^*P<0.05$ STZ + roflumilast versus STZ + vehicle). Data are shown as the relative mRNA expression normalized to GAPDH

the expression of NF-κB protein significantly increased in the DSM tissue from the rats in the STZ + vehicle group (0.82 ± 0.07, $n=6$, $N=6$) compared to that in the DSM tissue from the rats in control group (0.51 ± 0.06, $n=6$, $N=6$; $P<0.05$; Fig. 3a, b). Accordingly, the protein levels of TNF-α (0.68 ± 0.05, $n=6$, $N=6$), IL-6 (0.74 ± 0.07, $n=6$, $N=6$), and IL-1β (0.71 ± 0.08, $n=6$, $N=6$), which are modulated directly by NF-κB, were also higher in the DSM tissue from the rats in the STZ + vehicle group than in that from the rats in Con + vehicle group (0.37 ± 0.04; 0.33 ± 0.06; 0.29 ± 0.05, $n=6$, $N=6$; $P<0.05$; Fig. 3c–h).

Treatment with roflumilast abated the elevated levels of inflammatory cytokines in the DSM tissue of diabetic rats

Given that inflammation due to elevated levels of NF-κB, TNF-α, IL-6, and IL-1β is associated with impaired insulin signaling and deregulated glucose metabolism, PDE4 inhibitors should focus on its effects on inflammation. Our results indicate that the inflammatory factors induced by hyperglycemia are increased in diabetic rats. Interestingly, in the DSM tissue of diabetic rats, treatment with roflumilast was associated with a significant reduction in NF-κB gene expression (STZ + vehicle 0.79 ± 0.05, $n=16$, $N=7$; STZ + roflumilast 0.58 ± 0.04, $n=16$, $N=7$; $P<0.05$; Fig. 2a). Furthermore, treatment with roflumilast greatly reduced the elevated mRNA expression of TNF-α (STZ + vehicle 0.71 ± 0.06, $n=16$, $N=7$; STZ + roflumilast 0.51 ± 0.05, $n=16$, $N=7$), IL-6 (STZ + vehicle 0.69 ± 0.06, $n=16$, $N=7$; STZ + roflumilast 0.52 ± 0.05, $n=16$, $N=7$), and IL-1β (STZ + vehicle 0.65 ± 0.05, $n=16$, $N=7$; STZ + roflumilast 0.43 ± 0.04, $n=16$, $N=7$) in the DSM tissue of diabetic rats ($P<0.05$; Fig. 2b–d).

To further investigate the effects of roflumilast therapy on inflammation in DSM, we measured the protein expression of NF-κB, TNF-α, IL-6, and IL-1β, important inflammatory mediators implicated in bladder dysfunction. The protein levels of NF-κB (STZ + vehicle 0.82 ± 0.07, $n=6$, $N=6$; STZ + roflumilast 0.62 ± 0.05, $n=6$, $N=6$), TNF-α (STZ + vehicle 0.68 ± 0.05, $n=6$, $N=6$; STZ + roflumilast 0.47 ± 0.05, $n=6$, $N=6$), IL-6 (STZ + vehicle 0.74 ± 0.07, $n=6$, $N=6$; STZ + roflumilast 0.51 ± 0.06, $n=6$, $N=6$), and IL-1β (STZ + vehicle 0.71 ± 0.08, $n=6$, $N=6$; STZ + roflumilast 0.48 ± 0.06, $n=6$, $N=6$) were significantly lower in the PDE4 inhibitor-treated rats than in the STZ + vehicle-treated rats ($P<0.05$; Fig. 3a–h). Therefore, the pharmacological inhibition of PDE4 may play a primary role in inhibiting the release of inflammatory mediators in the DSM of diabetic rats.

Discussion

In recent years, the prevalence of diabetes has increased negatively affecting both individuals and society [20, 21]. During the progression diabetes, many complications can occur leading to further suffering. DBD is a common clinical

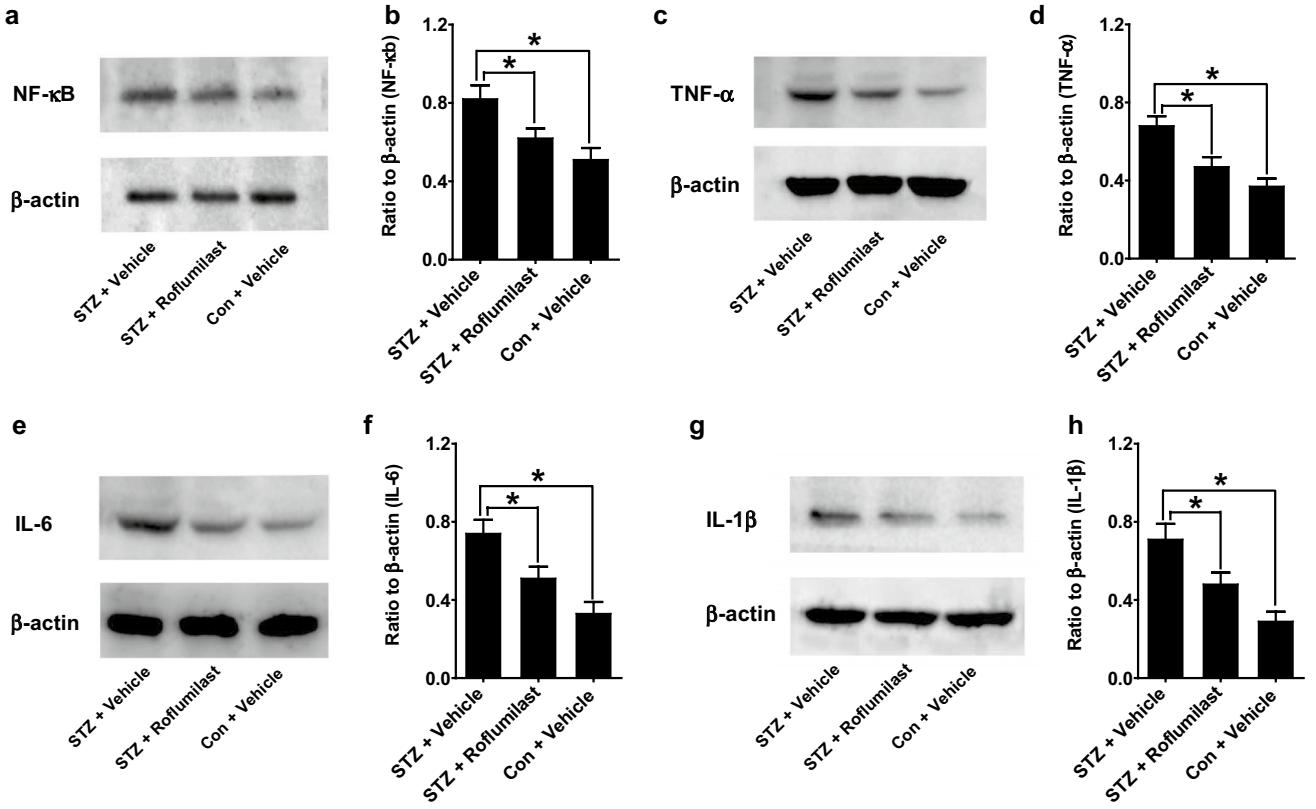

Fig. 3 Increased protein expression levels of inflammatory factors were deceased after oral roflumilast treatment in diabetic rat DSM. Comparison of the protein expression levels of NF-κB (**a, b**), TNF-α (**c, d**), IL-6 (**e, f**), and IL-1β (**g, h**) in the DSM. The protein levels of diabetic rats were significantly higher than those of the Con + vehicle group ($n=6$, $N=6$ for each group; $^*P<0.05$ Con + vehicle versus STZ + vehicle). The protein levels of the diabetic rats after oral roflumilast treatment were significantly decreased compared to those of the diabetic rats without treatment ($n=6$, $N=6$ for each group; $^*P<0.05$ STZ + roflumilast versus STZ + vehicle). Data are shown as the relative protein expression normalized to β-actin

syndrome that includes urinary storage and urination dysfunction. The symptoms related to the period of urine storage are particularly notable [22, 23]. However, the mechanism of DBD is not clear, and traditional treatments for DBD do not produce obvious benefits [24]. Therefore, we intend to seek a better therapeutic drug for treating DBD using a rat model of diabetes.

After the construction of the diabetes model, the blood glucose level was significantly higher in the diabetic rats than in the normal group, as indicated by tail vein blood measurements 3 days after STZ injection, which was consistent with the literature [17]. As many other studies have reported, at 6 weeks, body weights significantly decreased, whereas blood glucose levels and bladder weights were significantly increased in the STZ-induced diabetic rats compared to the values in the control group [25, 26]. The cystometry measurements in our present study indicated that bladder function was impaired, which mainly manifested as increases in the bladder capacity, voiding interval, voiding volume, and residual urine volume, which is consistent with the results of a previous study [25, 26]. These changes in diabetic rats are mainly associated with neuropathy and tissue remodeling [27]. Our previous studies have shown that PDE4 inhibitors can improve the bladder function of rats with high-fat diet-induced obesity [14]. At 6 weeks after STZ injection, the urinary dynamics results suggest that PDE4 inhibitors can alleviate DBD. However, the treatment with PDE4 inhibitors did not significantly ameliorate the blood sugar level in diabetic rats, as PDE4 inhibitors have no role in regulating blood glucose. Nevertheless, roflumilast, a selective PDE4 inhibitor, was effective in relieving DBD.

The literature indicates that diabetes leads to DBD via the following aspects: neurofactors, the DSM and bladder mucosa changes [28, 29]. Inflammatory and oxidative stress is very important in the changes in intracellular metabolism caused by hyperglycemia [30, 31]. Insulin resistance is a major pathophysiological change in metabolic syndrome, which is characterized by several low-level inflammatory states due to persistent activation of proinflammatory factors, including TNF-α, IL-6, IL-1β, and NF-κB [13, 32–34]. In this study, the expression of inflammatory factor protein and mRNA in the DSM tissue of diabetic rats was

significantly higher than that in the DSM tissue of normal rats. Inflammatory factors were therefore overexpressed in the untreated diabetic rats, causing damage to DSM tissue. In the comorbid condition of diabetes, increased inflammatory events are the major detrimental factors that impair insulin signaling and deregulate glucose metabolism [35, 36]. Inflammatory factors are reported to promote DNA and mitochondrial damage in DSM cells and induce DSM cell proliferation and fibrosis, leading to bladder dysfunction [37]. We can regard inflammatory factors as necessary factors for the bladder dysfunction caused by diabetes.

PDE4 inhibitors can significantly inhibit lipopolysaccharide-induced neutrophil recruitment in animal models [38]. Neutrophil chemotaxis is inhibited due to the inhibition of its potent chemokines IL-6 and TNF-α by PDE4 inhibitors [38]. Inflammatory cells secrete and may be involved in amplifying the inflammatory response by activating the transcription factor NF-κB, thereby increasing the expression of additional inflammatory genes. Roflumilast inhibits a variety of inflammatory signaling pathways, including the NF-κB pathway [39].Thus, PDE4 inhibitors are also therapeutically effective against the systemic inflammatory responses caused by diabetes. Oral roflumilast was established to relieve the inflammatory response to obesity-induced bladder dysfunction in the DSM of rats [14, 40]. Our present research also showed that the protein and mRNA expression of inflammatory mediators in the DSM tissue was significantly lower after 4 weeks of treatment with roflumilast than that in the diabetic group. The mRNA and protein expression levels of inflammatory factors found in the experiment proved that PDE4 inhibitors have therapeutic effects on the systemic inflammatory reaction in the DSM induced by diabetes. The trend for the protein and mRNA expression in the experiment is consistent and involves not only gene transcription but also a multichannel interaction. Therefore, the pharmacological inhibition of PDE4 has a therapeutic effect on diabetes-induced DSM inflammation and thus DBD.

Through these experiments, we demonstrated that a PDE4 inhibitor can ameliorate DBD by inhibiting the inflammatory reaction. These experiments are preliminary, and thus, we do not make a distinction between diabetes types; in subsequent research, we will observe the bladder changes in rat models of different types of diabetes. In this work, a DBD rat model was induced by STZ, and the effects of a PDE4 inhibitor on DBD were evaluated. The therapeutic effect of roflumilast on diabetes-induced DSM inflammation and DBD was confirmed, indicating that PDE4 inhibitors may represent a new direction for treating this disease in the future.

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Compliance with ethical standards

Conflict of interest Honglin Ding, Peng Zhang, Ning Li, Yili Liu, and Ping Wang declare that they have no conflict of interests.

Ethical approval All applicable International, National, and Institutional Guidelines for the care and use of animals were followed.

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