Activation of soluble guanylyl cyclase by BAY 58-2667 improves bladder function in cyclophosphamide-induced cystitis in mice

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BLADDER PAIN SYNDROME/INTERSTITIAL CYSTITIS (BPS/IC) is a complex chronic disease of unknown etiology with progressive development (21). Clinical symptoms for BPS/IC include chronic pelvic pain and pressure or discomfort related to bladder filling that is usually accompanied by other micturition symptoms, such as frequency, urgency, and nocturia (47). A number of hypotheses exist to explain BPS/IC, including damage and disruption of the bladder urethral barrier, local inflammatory reaction, and increased excitability of C-fibers (10, 31, 33). Current pharmacological treatments for BPS/IC usually aim to improve urinary bladder function and pain management include analgesic, anti-inflammatory, and immune suppressors, among others (21); however, most of these treatments present important side effects and are unsatisfactory.

Nitric oxide (NO), released from the urothelium or nerve fibers, influences efferent and afferent neurotransmission, resulting in inhibitory responses in the bladder smooth muscle (4, 5, 7). NO acts by enhancing the catalytic activity of the enzyme soluble guanylyl cyclase (sGC), a cytosolic, heterodimeric protein consisting of α- and β-subunits with a prosthetic heme group located in the β-subunit, which converts the nucleoside GTP into cGMP (16). The sGC heme group contains a central ferrous ion, which can be found in the reduced (Fe²⁺) or oxidized (Fe³⁺) forms, coordinated with four nitrogen atoms. This different state of the ferrous ion implies a redox regulation of enzyme activity, since the oxidation of the heme group or its loss leads to a NO-insensitive form of the enzyme (18). In the past decade, new pharmacological agents that target sGC/cGMP signaling directly were developed, comprising today two groups of drugs, namely, sGC stimulators and activators (38). Similarly to endogenous ligand NO, sGC stimulators such as BAY 41-2272 and BAY 63-2521 (Riociguat) increase sGC activity only when the heme ferrous ion is in a reduced state (Fe²⁺). In contrast, sGC activators such as BAY 58-2667 (cinaciguat) and BAY 60-2770 preferentially induce sGC activation when the heme ferrous ion is in its oxidized state (Fe³⁺) or missed (27, 36, 43). Inappropriate stimulation or NO insensitivity of sGC has been shown to account for the pathogenesis of vascular diseases associated with increased oxidative states, which is ameliorated by sGC activators (14, 29, 44, 46). Increased levels of oxidative stress promote oxidation and degradation of the sGC heme group, leading to an impairment of the NO-cGMP signaling pathway. Cystitis has been associated with decreased antioxidant mechanisms and accumulation of oxidative stress molecules (26, 34). We hypothesized that activation of sGC by BAY 58-2667 prevents cyclophosphamide (CYP)-induced cystitis through the enhancement of sGC activity and cGMP levels, consequently improving bladder function. Therefore, in the present study, we have evaluated the effects of BAY 58-2667 on voiding dysfunction, protein expressions of α₁ and β₁ sGC subunits, and cGMP levels in the bladder tissue after CYP exposure. The myeloperoxidase (MPO) activity and cyclooxygenase-2 (COX-2) protein expression as inflammatory markers, as well as reactive oxygen species (ROS) levels, were also evaluated in the bladder tissues.

MATERIALS AND METHODS

Animals. All animal procedures and experimental protocols were carried out according to the Ethical Principles in Animal Research.
adopted by the Brazilian College for Animal Experimentation and approved by the Institutional Committee for Ethics in Animal Research/University of Campinas (protocol number 3508-1). Eight- to 10-wk-old female C57BL/6 mice (20–25 g) were provided by Central Animal House Services of the University of Campinas. The animals were housed five per cage with a 12:12-h light-dark cycle. Food and water were available ad libitum.

Cystitis induction. Cystitis was induced with a single intraperitoneal injection of CYP at 300 mg/kg (Sigma, St. Louis, MO) or saline (10 ml/kg).

Treatments with BAY 58-2667 and experimental design. Mice were pretreated orally with 1 mg/kg of BAY 58-2667, i.e., [4-[[4-(carboxybutyl) -2-[[4-(phenethylbenzoyloxy)phenylenyl]amino]methyl][benzoic]acid] (Adipogen, Liestal, Switzerland), or its vehicle (5 ml/kg) Transcutol/Cremophor/water, 1:2:7, vol/vol (Sigma). After 1 h, mice were injected intraperitoneally with CYP or saline. Animals were assigned to four experimental groups, namely, Vehicle-Control (mice pretreated with vehicle and injected with saline); BAY 58-2667-Control (mice pretreated with BAY 58-2667 and injected with saline); Vehicle-CYP (mice pretreated with vehicle and injected with CYP); and BAY 58-2667-CYP (mice pretreated with BAY 58-2667 and injected with CYP). Experimental protocols were conducted at time 0 and 1, 2, 4, 6, 12, and 24 h after CYP (or saline) injection. For each time period, a number of 5–10 mice were used, unless otherwise stated. Doses of BAY 58-2667 and CYP were chosen according to previous studies (17, 41).

Histological analysis. Mice were weighed before and after every treatment. Bladders were excised, weighed, fixed in 4% paraformaldehyde, embedded in paraffin, sliced, and stained with hematoxylin and eosin for histological examination. For edema evaluation, bladders were excised, weighed, and a ratio between the wet and animal body weight was obtained.

Micturition pattern analysis of freely moving mice. Individual mice were placed in individual cages for urinary output collection on filter paper covering the cage bottom (17). First, urine was collected for 3 h for baseline measurement. Next, mice were pretreated with BAY 58-2667 (or vehicle) for 1 h and then injected with CYP or vehicle, as detailed above. Three hours before the end of treatments, each mouse was placed again in individual cages for another urine output measurement. Filter papers were removed, dried, photographed under UV light to visualize urinary spots, and analyzed with ImageJ software (National Institutes of Health, Bethesda, MD) to identify the surface area. The volumes of individual urine spots were calculated based on a calibration curve of fluid drops of known volume.

Cystometric study. Mice were anesthetized by intraperitoneal injection of urethane (1.8 g/kg). A 1-cm abdominal incision was made to expose the bladder and a 25-G cannula was inserted in the bladder dome. The cannula was connected to the infusion pump through a PE-50 catheter. Before cystometry was started, the bladder was emptied and contin-uous cystometry was performed by infusing saline in the bladder at 0.6 ml/h for 30 min after the end of the first micturition cycle. The following parameters were assessed: basal pressure, capacity (volume needed to induce first micturition), threshold pressure (intravesical pressure immediately before micturition), compliance (ratio between bladder capacity and threshold pressure), voiding pressure (pressure reached during micturition), frequency of voiding, intercontraction interval (ICI), and frequency of nonvoiding contractions (NVCs); spontaneous bladder contractions >4 mmHg from the baseline pressure that did not result in a voiding event (24). One mouse was used for each cystometrogram.

Functional assay in vitro. Mice were euthanized in a CO2 chamber. The bladders were immediately removed, and two longitudinal bladder smooth muscle strips with intact mucosa were obtained from each animal. Strips were mounted in 10-ml organ baths filled with Krebs-Henseleit solution (117 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM MgSO4, 1.2 mM KH2PO4, 25 mM NaHCO3, and 11 mM glucose, pH 7.4) continuously bubbled with a mixture of 95% O2 and 5% CO2. The temperature was maintained at 37°C by a thermoregu-lator water circuit. Each strip was connected to a transducer, and changes in isometric force were recorded using the PowerLab system, version 8 (ADInstruments, Sydney, AU). Resting tension was adjusted to 5 nN at the beginning of the experiments, each strip was allowed to equilibrate for 1 h, and during this time the Krebs-Henseleit solution was replaced every 15 min with fresh solution. After equilibration, cumulative concentration-response curves to the muscarinic receptor agonist carbachol (0.001–30 μM) were obtained. The strips were then washed with Krebs-Henseleit and allowed to return to baseline tension, equilibrated for 30 min, and then depolarized with 80 mM KCl in Krebs-Henseleit solution. Contractile response data were normalized to the wet weight of the respective bladder samples. The maximal response is shown in millinewtons per milligram wet weight.

Western blotting for α1- and β2-subunits of sGC and COX-2 in bladder tissue. After euthanasia, the bladders were immediately excised, weighed, and frozen in liquid nitrogen. Tissues were pulverized and homogenized in SDS lysis buffer for 30 min and then centrifuged (12,000 g, 4°C, 20 min). Protein concentrations of supernatants were determined by a Bradford assay, and equal amounts of protein from each sample were treated with Laemmli buffer containing 10% β-mercaptoethanol. Samples were heated in boiling water for 5 min and resolved by SDS-PAGE. The proteins were separated by 10% polyacrylamide gels and then electrotransferred to nitrocellulose membranes, performed for 90 min at a fixed amperage (0.15 mA/membrane) in a semidy device (Bio-Rad, Hercules, CA). Nonspecific protein binding was reduced by preincubation in blocking buffer (0.5% nonfat dry milk, 10 mM Tris, 100 mM NaCl, and 0.02% Tween 20). Detection using specific antibodies, horseradish peroxidase (HRP)-conjugated secondary antibodies, and luminal solution was performed. Anti-sGCα1 and anti-sGCβ1 were obtained from Novus Biologicals, anti-COX2 was obtained from Santa Cruz Biotechnology (Santa Cruz, CA), and anti-β-actin was from Sigma. Densitometry was performed using ImageJ software, and results are represented as the ratio of the density of analyzed protein to the density of the β-actin.

cGMP determination. Bladders were pulverized and subsequently processed according to the manufacturer’s protocol (Cayman Chemical, Ann Arbor, MI). The assays were performed in duplicate, normalized to the wet weight of the respective bladder, and expressed as picomoles per milligram of tissue.

Measurement of ROS. The oxidative fluorescent dye dihydroethidium (DHE) was used to evaluate in situ ROS generation. The bladders were embedded in a freezing medium, and transverse sections (12 μm) were obtained on a cryostate, collected on glass slides, equilibrated for 10 min in Hanks’ solution (1.6 mM CaCl2, 1.0 mM MgSO4, 145.0 mM NaCl, 5.0 mM KCl, 0.5 mM NaH2PO4, 10.0 mM glucose, 10.0 HEPES, pH 7.4). Fresh Hanks’ DHE solution (2 μM) was applied to each tissue section, and the slides were incubated in a light-protected humidified chamber at 37°C for 30 min. Images were obtained with a microscope (Eclipse 80i, Nikon, Tokyo, Japan) equipped for epifluorescence (excitation at 488 nm; emission at 610 nm) and a digital camera (DS-U3, Nikon). Fluorescence was detected with a 585-nm long-pass filter. The number of nuclei labeled with ethidium bromide in detrusor smooth muscle and urothelium wall was automatically counted using ImageJ software and is expressed as labeled nuclei per millimeter squared.

MPO assay. Bladders were pulverized, homogenized in hexadecyltrimethyl ammonium bromide buffer (HTAB; 0.5% HTAB in 50 mM potassium phosphate buffer, pH 5.4), and then centrifuged at 1,000 g, 4°C, for 5 min. The supernatant was used for an MPO assay through the reaction with 1.6 mM tetramethylbenzidine, 80 mM NaPO4, and 0.3 mM hydrogen peroxide. The protein concentration of each sample was measured by a Bradford assay. The absorbance was measured with a
spectrophotometer at 460 nm, and the results are expressed as optical
density (OD) per milligram of protein.

Statistical analysis. All measurements are expressed as means ±
SE of n experiments. Comparisons among the groups were evaluated
using one-way ANOVA, and the Tukey post hoc analysis (GraphPad
software). P < 0.05 was considered significant.

RESULTS

CYP-induced cystitis and sGC expression. Significant in-
creases in the bladder wet weight/animal body weight ratio
were observed in CYP-exposed mice within the 24-h exposure
compared with control mice (from 1.16 ± 0.07 to 2.18 ± 0.02
mg/g at 24 h; P < 0.05, n = 5, Fig. 1A). Histological evalua-
tion of bladder tissues revealed signs of edema of the lamina
propria, tissue disorganization, and urothelial denudation (Fig. 1B).

The protein expressions of both α1- and β1-subunits of sGC
in the bladder tissues were significantly reduced by ~40% in
the CYP-exposed group, as observed from 2 to 24 h after
exposure (n = 5, P < 0.05; Fig. 1C). Similar reductions in the
cGMP levels in the bladder tissues of the CYP group were seen
from 2 to 24 h compared with control mice (Fig. 1D). To
evaluate the effects of BAY 58-2667 on the in vivo functional
and blot analysis, the time point of 24 h of exposure
with CYP was routinely used.

Bladder dysfunction in CYP-exposed mice. We initially
studied the effects of pretreatment with BAY 58-2667
(1 mg/kg, 1 h) in freely moving mice using the micturition
pattern on filter paper (Fig. 2A). CYP-injected mice displayed
a significantly lower micturition volume compared with control
mice (0.024 ± 0.01 and 0.47 ± 0.08 ml, respectively, n = 7).

Fig. 1. Characterization of cyclophosphamide (CYP)-induced cystitis in mice and alterations of soluble guanylyl cyclase (sGC) expression and cGMP levels in
the bladder. A: bladder weight/animal body weight ratio in mice treated with CYP (300 mg/kg ip) during 24-h observation. B: histological bladder sections of
control and CYP-injected mice at 24 h depicting detrusor smooth muscle (DSM), submucosa (S), urothelium (U), and lumen (L). Representative Western
blots for sGC subunits (C) and levels of cGMP (D) after CYP administration at 24 h are shown. Blots were quantified by densitometry, and the expression of each
subunit was normalized for β-actin expression. Values are means ± SE; n = 5 mice/group; *P < 0.05 compared with time 0 (basal level). In B, yellow arrows
indicate urothelial denudation areas, and black arrows indicate submucosal/muscular edema.
A concomitant increase in the spot number by ~35-fold was also observed in the CYP group compared with control mice (161 ± 2.6 and 4.6 ± 1.5 spots, respectively; n = 7, Fig. 2, B and C). In CYP-exposed mice, BAY 58-2667 pretreatment significantly prevented the reduced micturition volume (0.09 ± 0.01 ml, n = 7) and attenuated the spot number (70.3 ± 14.1 spots, Fig. 2, B and C). In control mice, BAY 58-2667 affected neither the micturition volume (0.34 ± 0.03 ml, n = 5) nor the spot number (3.7 ± 1.8 spots, n = 5).

Cystometric recordings in anesthetized mice were also carried out in all groups (Fig. 3A). Considering all parameters evaluated, CYP-exposed mice displayed marked increases in basal pressure, voiding frequency, and NVCS, along with significant decreases in bladder capacity, compliance, and intercontraction interval (n = 7, Fig. 3, B–I). The threshold and micturition pressures did not significantly change between the control and CYP groups (n = 7, Fig. 3, D and F). All the cystometric alterations in the CYP group were significantly prevented by BAY 58-2667 pretreatment compared with vehicle-injected mice (n = 7, P < 0.05). In control mice, the only cystometric parameter significantly affected by BAY 58-2667 pretreatment was the basal pressure, which was reduced from 2.50 ± 0.36 ml in the vehicle group to 1.18 ± 0.21 ml in treated mice (n = 5, Fig. 3B).

Bladder contractility to carbachol of CYP-exposed mice. Addition of the muscarinic agonist carbachol (0.001–30 μM) produced concentration-dependent bladder contractions in all groups (Fig. 4A), but the maximal contractile responses were significantly reduced in the CYP compared with the control group (0.97 ± 0.14 and 2.15 ± 0.38 mN/mm, n = 6, respectively; Fig. 4B). Pretreatment with BAY 58-2667 in the CYP-exposed group significantly prevented the reduced carbachol-induced contractions (1.63 ± 0.28 mN/mm; n = 6, P < 0.05) without affecting the responses in the control group (1.81 ± 0.26 mN/mm, n = 5). In addition, the contractile responses by KCl (80 mM)-induced depolarization did not change significantly among groups (0.50 ± 0.03, 0.56 ± 0.06, 0.44 ± 0.06, and 0.43 ± 0.08 mN/mm for Vehicle-Control, BAY 58-2667-Control, Vehicle-CYP, and BAY 58-2667-CYP groups, respectively, n = 6; Fig. 4C).

Expression of sGC and cGMP levels in bladder tissues. Protein expression of α1- and β2-subunits of sGC and cGMP levels were significantly reduced in the bladder tissue of CYP compared with control mice (n = 7–10, P < 0.05). Pretreatment with BAY 58-2667 significantly prevented the reductions of the protein expressions of α1- and β2 sGC subunits after CYP exposure (n = 9, Fig. 5, A and B). The reduced cGMP levels in the CYP group were also fully prevented by BAY 58-2667 (n = 7, Fig. 5C). In the control group, no changes in sGC protein expression and cGMP levels by BAY 58-2667 were seen.

ROS generation in bladders of CYP-exposed mice. CYP exposure significantly increased the fluorescence intensity by 54% (P < 0.05) in detrusor smooth muscle (from 8.2 ± 0.5 to 12.6 ± 0.9 nuclei/mm², n = 5; Fig. 6, A and B). Similarly, in the urothelium the fluorescence intensity increased by 41% after CYP exposure (from 15.4 ± 1.2 to 21.7 ± 1.6 nuclei/mm², n = 5; Fig. 6, C and D). Pretreatment with BAY 58-2667 fully prevented increased ROS generation in both tissue sections of CYP-exposed animals (7.9 ± 0.9 and 14.4 ± 1.6 nuclei/mm² in detrusor and urothelium, respectively, n = 5). In the control group, no changes in ROS levels by BAY 58-2667 were seen.

Bladder edema, MPO activity, and COX-2 expression. The bladder wet weight/animal body weight ratio as a marker for inflammatory edema was significantly higher in the CYP group compared with control mice (n = 5, P < 0.05), but BAY 58-2667 had no significant effect on this parameter (Fig. 7A). Similarly, higher MPO activity and COX-2 protein expression were found in the CYP group (n = 5, P < 0.05), but both of
DISCUSSION

The present study demonstrated that CYP-induced micturition dysfunction in mice is accompanied by marked reductions of sGC expression and cGMP production in the bladder tissues, both of which are significantly prevented by pretreatment with the sGC activator BAY 58-2667.

BPS/IC is a long-recognized syndrome with a profound negative impact on quality of life of patients. The exact pathogenic mechanisms of this disease remain as yet to be determined.

Fig. 3. Representative cystometric traces of a single animal per group (A) and relative changes in cystometric parameters (B–G) in control and CYP (300 mg/kg)-injected mice, pretreated or not with BAY 58-2667 (1 mg/kg, gavage). Arrows indicate micturition peaks. Values are means ± SE; n = 5–7 mice/group; *P < 0.05 vs. Vehicle-Control. +P < 0.05 vs. BAY 58-2667-Control; #P < 0.05 vs. Vehicle-CYP.

Fig. 4. Concentration-response curves to carbachol (0.001–30 μM; A) and maximal responses for this agonist (B) in the bladders of control and CYP (300 mg/kg)-injected mice, pretreated or not with BAY 58-2667 (1 mg/kg, gavage). C: Isometric contractile responses to KCl depolarization (80 mM). Values are means ± SE; n = 5–6 mice/group.*P < 0.05 vs. Vehicle-Control. #P < 0.05 vs. Vehicle-CYP.
clarified, and treatment regimens are mainly symptomatic. Bladder inflammation induced by CYP in rodents is a well-established experimental model for BPS/IC (6). After systemic administration, CYP is partly metabolized to acrolein, which accumulates in the bladder causing local irritation, resulting in severe inflammation and bladder hyperreflexia (9). Because there are many similarities between BPS/IC and CYP-induced cystitis in rodents, this model has widely been used to characterize the inflammatory pathways, functional alterations, and to identify potential novel therapeutic targets for this disease (13). Within 24 h after a single CYP administration in rats and mice, an acute inflammation in the bladder is observed, characterized by loss of barrier integrity, urothelial cell apoptosis, and mucosal edema, along with cell infiltration (neutrophils, monocytes, and mast cells) (1, 22, 42). Accordingly, in our study, cystitis was successfully induced by a single CYP injection in

Fig. 5. Pretreatment with BAY 58-2667 (1 mg/kg) prevents sGC degradation and normalizes the cGMP levels in bladder tissues of CYP (300 mg/kg)-injected mice. A and B: representative Western blots for sGC α₁ (A)- and β₁ (B)-subunits. Blots were quantified by densitometry, and the expression of each subunit was normalized for β-actin expression. Values are means ± SE; n = 7–10 mice/group. *P < 0.05 vs. Vehicle-Control. #P < 0.05 vs. Vehicle-CYP.

Fig. 6. Reactive oxygen species (ROS) generation in bladders of CYP (300 mg/kg)-exposed mice, pretreated or not with BAY 58-2667 (1 mg/kg, gavage). Representative images of ROS measurement through dye dihydroethidium-induced fluorescence in the detrusor smooth muscle (DSM; A) and the urothelium (C) are shown. Quantification of ethidium bromide-positive nuclei in DSM and urothelium are shown in B and D, respectively. Values are means ± SE; n = 5 mice/group. *P < 0.05 vs. Vehicle-Control. #P < 0.05 vs. Vehicle-CYP. Scale bar = 100 μm. Magnification, ×200.
mice, as evidenced by inflammatory edema, urothelial disruption, and bladder dysfunction. Although there are histopathological bladder differences between 24-h CYP administration in mice and chronic BPS/IC, a widespread destruction of the urothelium and bladder wall irritation thus leading to urgency, dysuria, frequency, and nocturia are common features seen in both conditions (47). In agreement with previous urodynamic studies (17, 49), CYP-exposed mice underwent marked changes toward a dysfunctional hyperactive phenotype. The voiding behavior in freely-moving mice was characterized by a large number of urinary spots and small urinary volumes, which is consistent with the cystometric recordings showing increases in micturition frequency, NVCs, and basal pressure. The urinary bladder is densely innervated by parasympathetic nerve fibers, the activation of which results in the release of acetylcholine, which produces detrusor smooth muscle contractions via muscarinic receptor activation (3). A predominance of the muscarinic M2 receptor subtype and a minor population of muscarinic M3 receptors has been reported in the urinary bladder; however, the M3 subtype is the most important to mediate the cholinergic contractile responses, whereas the role of the M2 subtype in the bladder remains unclear. Studies have shown that the expression and functional roles of muscarinic receptors in the bladder may be altered in the course of cystitis (19, 23, 28, 32). Immunoblotting and immunohistochemistry revealed that the alterations in the cholinergic system by CYP exposure involve an upregulation of muscarinic M2, M4, and more prominently M5 receptors in the urothelium (19). Recently, a newly described laser-capture microdissection method to separate urothelial cells from detrusor cells was employed to evaluate the expressions of muscarinic M2 and M3 mRNA in CYP-induced chronic cystitis in rats (45). The mRNA expressions of both of these muscarinic receptors increased in urothelial cells and decreased in detrusor muscles. In our study, the in vitro bladder contractions induced by the muscarinic agonist carbachol were reduced in CYP-exposed mice, which is consistent with previous studies (4, 20, 26). Our findings that KCl (80 mM)-induced contractions remained unchanged between control and CYP groups indicate that the bladder contractile capacity is not detrimentally affected by cystitis, reinforcing rather that the cholinergic system/receptors are affected by CYP exposure. It has been suggested that increased expression of urothelial muscarinic M2 and M3 receptors contributes to enhanced interactions of urothelially released acetylcholine with these receptors, which result in activation of afferent pathways, leading to bladder dysfunction associated with bladder inflammation (45). Moreover, the reduced detrusor contractile force to muscarinic receptor activation in CYP-exposed mice may account for the smaller urine volumes that result in higher residual urine associated with increased micturition frequency. The apparent discrepancy between the overactive bladder profile (urodynamic studies) and the reduced detrusor contractility in vitro maybe rely on an excessive sensitization of afferent pathways and release of urothelial-derived factors (8, 19). It has also been proposed that conditions of severe inflammation and oxidative stress might cause detrusor underactivity due to smooth muscle damage (34).

Activators of sGC, including BAY 58-2667, interact directly with the prosthetic heme group of sGC, enhancing the enzyme responsiveness in oxidative conditions (29, 40, 44). In conditions of NO blockade, systemic activation of sGC by BAY 58-2667 was recently shown to normalize hypertention and renal vasoconstriction, restoring renal blood flow (14). In our study, BAY 58-2667 treatment in CYP-exposed mice significantly prevented the reductions in the voided volume and the spot number in freely moving mice, as well as the cystometric alterations, including the increases in basal pressure, voiding frequency, and NVCs, and the decreases in capacity, intercontraction intervals, and compliance in anesthetized mice. The reduced bladder contractions to carbachol in the CYP group were also prevented by BAY 58-2667. We next moved to explore the mechanisms by which a sGC activator like BAY 58-2667 prevents in vivo and in vitro bladder dysfunction of CYP-induced cystitis, and performed immunoblotting of sGC subunits and measured the cGMP levels in the bladders. We found that CYP exposure resulted in marked reductions of expressions of α1- and β1-sGC subunits and cGMP production, both of which were fully prevented by BAY 58-2667 pretreatment. Increased oxidative stress has been implicated in different pathological conditions in the urinary bladder (12, 15, 48), including experimental cystitis (23, 26, 28, 35). The oxidative stress accompanying type II diabetes also deteriorates urethral smooth muscle function in mice (2). Moreover, in oxidative stress conditions, the sGC redox state in blood vessels is altered to an oxidized state, possibly as a consequence of the large generation of ROS species such as superoxide, leading to protein loss/degradation and damage to cellular structure and function.
function (30, 44). To evaluate the levels of oxidative stress in bladder tissues of CYP-exposed mice, we performed DHE imaging of fresh frozen sections of bladders, identifying the ROS formation in the detrusor and urothelium layers. Our data showed that CYP exposure significantly increases the ROS levels in both detrusor and the urothelium, which is prevented by BAY 58-2667. Therefore, ROS generation in the course of CYP-induced cystitis is likely to contribute to sGC degradation, and BAY 58-2667, by stabilizing the prosthetic heme group of sGC, prevents enzyme degradation, rescuing the enzyme responsiveness in oxidative conditions. We observed a marked inflammation accompanying the bladder dysfunction by CYP exposure, as evidenced by the increases in MPO activity, COX-2 expression, and the bladder wet weight/animal body weight ratio. However, BAY 58-2667 failed to affect these parameters, suggesting that amelioration by this sGC activator on voiding function takes place independently of the local inflammatory process. At the dose used in the present study, BAY 58-2667 did not significantly interfere with most of the functional and molecular assays in the noninflamed (control) group, reinforcing that BAY 58-2667 actions rely on an oxidative condition. We may not ignore, however, that BAY 58-2667 pretreatment in the control mice significantly reduced the basal pressure (cystometry), which is suggestive that BAY 58-2667 itself produces some level of bladder relaxation.

In BPS/IC patients, a previous study demonstrated that bladder blood flow during filling is reduced, despite less clarity as to whether this alteration partly or totally accounts for bladder symptoms (37). Recognized as a vasodilator, BAY 58-2667 is capable of producing sustained vasodilation in heart and pulmonary vessels, improving tissue perfusion and reducing injury (11, 39). BAY 58-2667 therefore may improve bladder function through an indirect effect by promoting vasodilatation in vessels supplying the bladder, thus facilitating the clearance of reactive species.

In summary, the current study demonstrates that pretreatment with BAY 58-2667 significantly prevents micturition dysfunction in CYP-induced mouse cystitis. The preservation of sGC subunits and normalization of cGMP production resulted in amelioration of bladder dysfunction and maintenance of adequate micturition cycles. The lack of accurate biomarkers creates difficulties in diagnosis and development of effective pharmacological strategies for BPS/IC. Future research is required for a better understanding of the pathophysiology of this disease and identification of novel, safe, and effective therapeutic options. The class of sGC activators may provide a novel option to treat BPS/IC.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

REFERENCES


