Full Paper

A Novel Mouse Model of Chronic Inflammatory and Overactive Bladder by a Single Intravesical Injection of Hydrogen Peroxide

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Abstract. There is so far no generally accepted animal model of chronic cystitis by which potential therapies can be evaluated. In this study, we aimed to establish a new mouse model of cystitis based on the proinflammatory effects of reactive oxygen species. A single intravesical injection of 1.5% hydrogen peroxide (H2O2) significantly increased the numbers of voids by 1 day after injection in female mice, which lasted up to 7 days. The H2O2 injection rapidly increased the bladder weight by 3 h in parallel with the histological damage and hyperpermeability of urothelial barrier. Although the urothelial dysfunction was recovered to normal by 7 days, increase in bladder weight, edematous thickening of the submucosa, and vascular hyperpermeability were apparent even 7 days after injection. During the time course, massive infiltration of neutrophils and increased expression of inflammatory cytokines were observed in the bladder. An intraperitoneal administration of oxybutynin, amitriptyline, indomethacin, or morphine attenuated the H2O2-induced frequent urination. These findings suggest that an intravesical injection of H2O2 induces relatively long-lasting inflammatory and overactive bladder, compared with existing cystitis models. The intravesical H2O2 injection model may be a simple and useful tool in the pathological study and drug discovery for chronic cystitis.

[Supplementary Figure: available only at http://dx.doi.org/10.1254/jphs.12265FP]

Keywords: chronic cystitis, hydrogen peroxide, interstitial cystitis, overactive bladder, long-lasting

Introduction

Chronic inflammation in the urinary bladder generally causes irritative voiding symptom (urinary frequency, urgency, and nocturia) and pelvic or lower abdominal pain (1, 2). Chronic inflammatory bladder diseases, such as interstitial cystitis (IC), are considered to be due to infectious and noninfectious etiology, including medication, radiation, toxic urinary agents, autoimmune response, urothelial dysfunction, and neurogenic causes, which could be responsible for the induction of inflammatory responses (3, 4). However, there are little or no reliably effective therapies and drugs for IC and/or the symptoms of IC in the world (5 – 7). The main reason for this is that there is almost no generally accepted animal model of chronic cystitis by which potential therapies or drugs can be evaluated.

The most commonly used cystitis model is cyclophosphamide (CP)-induced cystitis, in which intraperitoneal (i.p.) injection of CP induces acute hemorrhagic cystitis, accompanied with acute inflammation, bladder overactivity, and pain-related behaviors (8 – 11). Furthermore, there are a variety of experimental cystitis animal models induced by ifosfamide (12, 13), acrolein (13 – 15), mustard oil (16, 17), acetic acid/protamine sulphate (18), or lipopolysaccharide/protamine sulphate...
(19). However, these cystitis models are characterized by acute inflammation, rather than chronic pathologic features; the duration of inflammation and bladder overactivity in these models is short (within several days), which disagrees with the pathology of chronic inflammatory bladder diseases such as IC. Therefore, chronic cystitis animal models showing more long-lasting inflammatory and overactive bladder are needed to elucidate the pathology and drug discovery for the diseases.

Accumulating evidence suggests that reactive oxygen species (ROS) such as hydrogen peroxide \((H_2O_2)\) play a critical role in cystitis. CP-induced cystitis is caused by the metabolite acrolein, which rapidly enters into the urothelial cells and produces ROS, and is prevented by ROS scavengers or antioxidants (13, 20 – 22). It is suggested that bladder dysfunction induced by bladder outlet obstruction (23, 24) and ischemia/reperfusion (25, 26) are due to the generation of ROS. In addition, ROS are abundant in the bladder, generated by infiltrated inflammatory cells such as macrophages, neutrophils, and mast cells in the inflamed bladder (27 – 29). Thus, excess ROS generated endogenously can lead to lipid peroxidation, protein oxidation, and DNA damage in the bladder. Recent studies demonstrate that an intravesical injection of \(H_2O_2\) causes immediately detrusor overactivity in anesthetized rats, suggesting ROS show the stimulatory effect on the bladder afferent fibers directly and/or indirectly (30, 31). However, the long-term effects of an intravesical injection of \(H_2O_2\) on structural and functional changes of the bladder have not been clarified. In the present study, to establish a new mouse model of cystitis based on the proinflammatory effects of ROS, we have determined whether a single intravesical injection of \(H_2O_2\) causes bladder inflammation and overactivity in female mice.

**Materials and Methods**

**Animals**

Female C57BL/6J mice aged 5 – 6 weeks were purchased from Japan SLC, Inc. (Hamamatsu). They were housed and bred in groups of 4 – 6 per cage in a room maintained at 24°C ± 1°C and 35% – 75% relative humidity with an alternating 12-h light/dark cycle (the lights came on automatically at 8:00 a.m.). Food and water were freely given. All animal care and experimental procedures were in accordance with the ethical guidelines of the Kyoto University Animal Research Committee; the Internal Regulations on Animal Experiments at Nippon Shinyaku Co., Ltd., which are based on the Law for the Humane Treatment and Management of Animals (Law No. 105, 1 October 1973, as amended on 1 June 2006); and Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society.

**Drugs**

\(H_2O_2\) was purchased from Santoku Chemical Industries, Ltd. (Tokyo). CP monohydrate and catalase, a \(H_2O_2\) scavenger, were from Wako Pure Chemical Industries, Ltd. (Osaka). \(H_2O_2\), CP, and catalase were dissolved in sterile saline (Otsuka, Tokushima). Oxybutynin chloride, an anticholinergic drug, was from Sigma-Aldrich Japan (Tokyo). Amitriptyline hydrochloride, a tricyclic antidepressant, was from ZereneX Molecular, Ltd. (Manchester, UK). Indomethacin, a potent non-steroidal anti-inflammatory drug (NSAID), was from Nacalai Tesque, Inc. (Kyoto). Morphine hydrochloride, an opioid analgesic, was from Takeda Chemical Industries (Osaka). Amitriptyline, indomethacin, oxybutynin, and morphine were dissolved in 5% DMSO and 2% Tween80 (Nacalai Tesque) in saline. Drugs were prepared immediately before use.

**Induction of \(H_2O_2\)- and CP-induced cystitis**

For \(H_2O_2\)-induced cystitis, under isoflurane anesthesia, a 24-G polypropylene catheter was introduced into the bladder until urine was observed through the catheter, and the lower abdomen was pushed slightly until the urine was eliminated. Then, 50 \(\mu\)L of 0.5% – 5% \(H_2O_2\) solution dissolved in saline solution was introduced into the bladder through the catheter. The \(H_2O_2\) solution was introduced again into the bladder 30 min later to confirm contact with the bladder urothelium. For CP-induced cystitis, CP (300 mg/kg) or saline was administered intraperitoneally.

**Measurement of number of voids and bladder weight**

Three hours, 1 day, and 7 days after intravesical injection of \(H_2O_2\) or i.p. injection of CP, mice were allowed to acclimate to laboratory conditions for 30 min before treatment in an individual clear plastic cage (20 × 25 × 14 cm: width × length × height) lined with filter paper (Advantec Chromatography Paper No. 50; Toyo Roshi Kaisha, Ltd., Tokyo). After a new piece of filter paper had been inserted, the mouse was videotaped for 15 min. The number of voids was quantified afterwards from the videotape by counting the number of spots on the filter paper. After observation, the mice were killed by cervical dislocation. The bladders were then removed and blotted dry and their wet weight was measured. To assess the effect of catalase, catalase (3,000 U) was administered intravesically 30 min before or immediately after the intravesical injection of 1.5% \(H_2O_2\) solution. Amitriptyline (1 mg/kg), indomethacin (3 mg/kg), oxy-
butynin (3 mg/kg), morphine (3 mg/kg), or saline in a volume of 10 mL/kg were administered intraperitoneally 7 days after the intravesical injection of 1.5% H$_2$O$_2$ solution. Thirty minutes after the drug administration, the number of voids and bladder weight were measured.

**Measurement of bladder urothelial and vascular permeabilities**

The mice were injected via the bladder or the tail vein through the catheter with 0.05 mL or 0.1 mL of 5% Evans blue (Nacalai Tesque) in sterile normal saline, respectively. Thirty minutes after the injection, mice were killed by cervical dislocation and the bladders were removed. Bladder vascular or urothelial membrane permeabilities were assessed by extracting the Evans blue from the bladder by heating (56°C) in formamide (1 mL/bladder) overnight, and then measuring the dye concentration (μg/mL) spectrophotometrically (Bio-Rad, Hercules, CA, USA).

**Histological examination and immunohistochemistry**

Mice were deeply anesthetized with sodium pentobarbital and perfused transcardially with potassium-free phosphate-buffered saline (PBS), followed by 4% paraformaldehyde. The bladders were removed, and split longitudinally. The bladder tissues were postfixed overnight in 4% paraformaldehyde and embedded in paraffin. The paraffin-embedded tissues were cut into 5-μm sections, and then stained with hematoxylin and eosin (HE) with the standard procedures. Gross histologic observations were performed by using a microscope system (BZ-8100; Keyence, Osaka).

For immunohistochemistry against Gr-1, a marker of neutrophils, deparaffinized sections were treated with hydrogen peroxide to quench endogenous peroxidase and blocked with bovine serum albumin (BSA) for 1 h. Slides were then incubated with the primary antibodies for Gr-1 (rat anti-Gr-1/Ly-6G antibody, 1:300; R&D Systems, Minneapolis, MN, USA) at 4°C overnight. Immunohistochemistry followed the peroxidase method with biotinylated rabbit anti-rat IgG (1:200; Vector Laboratories, Burlingame, CA, USA), ABC kit (1:200, Vector Laboratories), and diaminobenzidine (Dojindo, Kumamoto) as chromogen. Thereafter, counterstaining was performed with hematoxylin.

**Real-time RT-PCR**

The removed bladders were flash-frozen in liquid nitrogen and stored at −80°C until use. Total RNA from the tissues was isolated with ISOGEN reagent (Nippon Gene, Tokyo) and cDNA was synthesized with ReverTra Ace qPCR RT Kit (Toyobo, Osaka). Real-time quantitative PCR was performed using the StepOne real-time PCR system (Life Technologies, Carlsbad, CA, USA) in a final volume of 20 μL containing 1.0 μg of total RNA with THUNDERBIRD SYBR qPCR Mix (Toyobo). The oligonucleotide primers used for β-actin were 5′-AGTGTGACGTTGACATCCGTGA-3′ and 5′-GCC AGAGCAGTAATCTCCTCTTCT-3′; for tumor necrosis factor (TNF)-α, 5′-TGCTATGTCTACGCCCTTCT-3′ and 5′-GAGGCCATTGGGAACCTTCT-3′; for interleukin (IL)-1β, 5′-TCCAGTGAGACATGAGCAC-3′ and 5′-GAAAGTCACACACCAGCGTTA-3′; for IL-6, 5′-GCTACAAACTGGATATAATCAGGA-3′ and 5′-CCAGGTAGCTATGTGACTCCAGAA-3′. The results for each gene were normalized relative to β-actin levels measured in parallel in each sample.

**Statistical analyses**

Data from all experiments are presented as means ± S.E.M. and were analyzed with the SAS program (Ver. 8.2; SAS Institute Inc., Cary, NC, USA). Statistical analysis was performed with one-way analysis of variance (ANOVA), followed by the Newman-Keuls post-hoc comparison test. The mRNA expression levels of inflammatory cytokines were analyzed by two-way ANOVA, followed by Bonferroni’s post-hoc comparison test. In all cases, a P-value of less than 0.05 was considered statistically significant.

**Results**

**Number of voids and bladder weight in H$_2$O$_2$- or CP-injected mice**

The effects of a single intravesical injection of H$_2$O$_2$ or a single i.p. injection of CP on the number of voids were examined 3 h and 1, 7, and 14 days after the injection (Fig. 1). An intravesical injection of 0.5% H$_2$O$_2$ had no effect on the number of voids at any times tested ($F_{4,20} = 1.58$, $P = 0.226$). The intravesical injections of 1.5% and 5% H$_2$O$_2$ significantly increased the number of voids ($F_{4,21} = 5.88$, $P < 0.01$ and $F_{4,21} = 4.77$, $P < 0.01$, respectively). The significant increases were observed 1 day after and lasted 7 days after the injection in both doses, compared with that in non-injected control mice, while they disappeared within 14 days after the injection. An i.p. injection of CP (300 mg/kg) significantly increased the number of voids ($F_{4,23} = 28.62$, $P < 0.001$). However, the significant increase was observed only 3 h after the injection, compared with that in control mice. The increase was decreased 1 day after and fully disappeared 7 and 14 days after the injection.

Similarly, the effects of a single intravesical injection of H$_2$O$_2$ or a single i.p. injection of CP on the number of voids were examined 3 h and 1, 7, and 14 days after the injection (Fig. 2). An intravesical injection of 0.5%
H₂O₂ slightly, but significantly increased the bladder weight \((F_{3,20} = 16.10, \ P < 0.001)\). The significant increases were observed 3 h and 1 day after the injection, compared with that in control mice, while the increase disappeared within 7 days after the injection. The intravesical injections of 1.5% H₂O₂ significantly increased the bladder weight \((F_{4,22} = 20.29, \ P < 0.001)\). The significant increases were observed 3 h and lasted 14 days after the injection, although the increase tended to decrease. The intravesical injections of 5% H₂O₂ increased the bladder weight \((F_{4,23} = 2.84, \ P = 0.05)\), while the significant increase was observed only 7 days after the injection. However, because the survival rate in 5% H₂O₂-injected mice 7 days after the injection was low \((7/12)\), we could not analyze correctly the number of voids and bladder weight. Therefore, we have selected a single intravesical injection of 1.5% H₂O₂ in the following examinations. On the other hand, an i.p. injection of CP (300 mg/kg) significantly increased the bladder weight \((F_{4,23} = 28.83, \ P < 0.001)\), and the
significant increases were observed only 3 h and 1 day after the injection, compared with that in control mice. The increase in bladder weight was recovered 7 and 14 days after the CP injection.

Effects of catalase on the increased number of voids and bladder weight in H2O2-injected mice

Catalase (3000 U/50 μL) was injected intravesically (i.ves.) 30 min before (pre) or immediately after (post) the intravesical injection of 1.5% H2O2 or saline. After 7 d, the number of voids was counted for 15 min (A), and then the bladder was removed and weighed (B). Values are means ± S.E.M. for a group of 3 – 9 mice. *P < 0.05, **P < 0.01, compared with intravesical saline-injected group. *P < 0.05, **P < 0.01, compared with intravesical H2O2-injected group without catalase.

Bladder urothelial and vascular permeabilities in H2O2-injected mice

The effect of an intravesical injections of 1.5% H2O2 on the bladder urothelial and vascular permeabilities were examined by Evans blue extracting. When the mice were injected intravesically with Evans blue for assessment of the bladder urothelial permeability, the dye concentration in the bladder was significantly increased (F(3,23) = 32.23, P < 0.001). The significant increases were observed 3 h and 1 day after the H2O2 injection, compared with that in control mice, but it was recovered to the control level 7 days after the injection (Fig. 4A). When the mice were injected with Evans blue via the tail vein, respectively, 30 min before the indicated time. The bladders were removed, and the dye concentrations (μg/mL) were measured. Values are means ± S.E.M. for a group of 4 – 10 mice (A) and 6 mice (B). ***P < 0.001, compared with non-injected control mice.

Histopathological and immunohistochemical analysis of the bladder in H2O2-injected mice

The cystitis induced by the intravesical injections of
Fig. 5. Histopathological analysis of the bladder in H2O2-injected mice. Mice were injected intravesically (i.ves.) with saline (A – C) or 1.5% H2O2 (D – F). After 3 h (A, D), 1 day (B, E), or 7 days (C, F), the bladders were removed, and tissue sections (5 μm) were stained with hematoxylin and eosin. Arrowheads indicate thin or destroyed areas of the bladder urothelium. Asterisks indicate the bladder lumen. Scale bar = 200 μm.

Fig. 6. Representative images of immunostaining for the neutrophil marker Gr-1 in H2O2-injected mice. Mice were injected intravesically (i.ves.) with saline (A) or 1.5% H2O2 (B). After 7 d, the bladders were removed, and tissue sections (5 μm) were immunostained with Gr-1 antibody. Thereafter, the sections were counterstained with hematoxylin. Scale bar = 50 μm. A’, B’) Enlarged images are shown in rectangles. Scale bar = 20 μm. Arrowheads indicate Gr-1–positive cells. Asterisks indicate the blood vessels.
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1.5% H₂O₂ was histopathologically examined by HE staining for evidence of altered bladder morphology (Fig. 5). In saline-injected mice, weak edematous thickening was observed in the submucosa 3 h and 1 day after the injection, probably due to the pressure of saline solution to the inner wall of the bladder. The edematous thickening in saline-injected mice was recovered to normal by 7 days after the injection. In H₂O₂-injected mice, the bladder urothelium was thin or destroyed, and severe edematous thickening was observed in the submucosa 3 h and 1 day after the injection, compared with saline-injected mice. The damage of the bladder urothelium was recovered to normal by 7 days after the injection, while severe edematous thickening of the submucosa was apparent even 7 days after the injection. Furthermore, a large number of inflammatory cells, vascularization and hyperplasia were observed in the submucosa.

To examine the effect of an intravesical injection of H₂O₂ on neutrophil infiltration, we immunohistochemically examined the distribution of Gr-1-positive cells, a neutrophil marker, in the bladder sections. In saline-injected mice, only a few Gr-1-positive cells were observed in the submucosa. In H₂O₂-injected mice, a large number of Gr-1-positive cells were observed mainly in the submucosa 7 days after the injection (Fig. 6).

Expression of inflammatory cytokines in the bladder of H₂O₂-injected mice

We measured the mRNA expression levels of TNF-α, IL-1β, and IL-6 by real-time PCR. The intravesical injection of H₂O₂ significantly elevated the mRNA expression levels of TNF-α (two-way ANOVA, saline × H₂O₂; F₁,₁₈ = 45.87, P < 0.001), IL-1β (two-way ANOVA, saline × H₂O₂; F₁,₁₈ = 45.87, P < 0.001) and IL-6 (two-way ANOVA, saline × H₂O₂; F₁,₁₈ = 23.82, P < 0.001). The significant elevations of TNF-α mRNA expression were observed 3 h and 7 days after the injection, compared with that in saline-injected mice (Fig. 7A). The significant elevation of IL-1β mRNA expression was observed 1 day after the injection and tended to be sustained for 7 days (Fig. 7B). The significant elevations of IL-6 mRNA expression were observed 3 h and 1 day after the injection, while it decreased with time (Fig. 7C).

Effect of the therapeutic drugs on the increased number of voids, bladder weight, and TNF-α mRNA expression induced by H₂O₂ injection

For the current therapy for IC and overactive bladder syndrome, anticholinergic drugs, tricyclic antidepressants, and NSAIDs are used as medical treatment to relieve bladder hyperactivity and pain (6, 7, 32, 33). Opioids may be used for severe bladder pain and referred pain in IC patients (34). Seven days after the H₂O₂ injection, a single i.p. administration of oxybutynin (3 mg/kg), amitriptyline (1 mg/kg), indomethacin (3 mg/kg), or morphine (3 mg/kg) 30 min before testing significantly attenuated the increased number of voids, compared with the i.p. vehicle–administered group (Fig. 8A). On the other hand, the increased bladder weight was not changed by the treatment with these drugs (Fig. 8B). An i.p. administration of oxybutynin and indomethacin had no effect on the elevation of TNF-α mRNA expression. Although amitriptyline and morphine tended to decrease it, no significant difference was observed, compared with the vehicle-administered group (Supplementary Fig. 1: available in the online version only).
Discussion

In the present study, we established the H$_2$O$_2$-induced cystitis model showing relatively long-lasting bladder inflammation and overactivity in mice, compared with the existing cystitis models, as supported by the following findings: i) A single intravesical injection of H$_2$O$_2$ increased the number of voids by 1 day, and it lasted up to 7 days after the injection, which was delayed but longer than that in CP-induced cystitis model. ii) The H$_2$O$_2$ injection rapidly increased the bladder weight, which was paralleled with histological damage and hyperpermeability of the urothelial barrier. Although the urothelial dysfunction was recovered to normal by 7 days, increased bladder weight, edematous thickening of the submucosa, and vascular hyperpermeability lasted up to 7 days after the injection. iii) The H$_2$O$_2$ injection induced massive infiltration of neutrophils and elevation of the mRNA expression levels of inflammatory cyto-

kines, suggesting bladder inflammation. iv) A single administration of the anticholinergic drug, tricyclic antidepressant, NSAID, or opioid analgesic suppressed the H$_2$O$_2$-induced urinary frequency.

Accumulating evidence suggests that ROS play a role in the pathogenesis of urinary dysfunction (23 – 25, 29, 35, 36). CP-induced cystitis is caused by the metabolite acrolein, which rapidly enters into the urothelial cells, where it then activates intracellular ROS and nitric oxide production leading to peroxynitrite production, and the increased peroxynitrite damages bladder tissues (20). Recent studies in lower urinary tract symptom (LUTS) patients suggest that the obstruction of a bladder urothelial membrane and an increase of oxidative stress followed a bladder ischemia and inflammation may cause urinary dysfunction (37, 38). Furthermore, it has been shown that that an intravesical administration of H$_2$O$_2$ evokes immediately detrusor overactivity in anesthetized rats by continuous cystometrography, and oxidative stress induced by H$_2$O$_2$ sensitizes the capsaicin-sensitive C-fiber afferent pathway, thereby inducing detrusor overactivity (30, 31). The present findings further support the hypothesis that ROS play a key role in the generation of inflammatory and overactive bladder.

In the present study, the H$_2$O$_2$-evoked bladder inflammation and overactivity were almost abolished by the pretreatment with catalase, a H$_2$O$_2$ scavenger. However, it is unlikely that exogenous H$_2$O$_2$ mediates directly delayed overactive bladder observed in our model because the removal of exogenous H$_2$O$_2$ by post-treatment with catalase had no effect. Therefore, it is considered that the long-lasting bladder inflammation and overactivity are triggered by rapid and transient insult to the bladder wall during intravesical H$_2$O$_2$ infusion. H$_2$O$_2$ exogenously injected into the bladder causes acute damage to bladder urothelial cells probably by lipid peroxidation, protein oxidization, and DNA damage, leading to hyperpermeability of the urothelial barrier. Thus, the submucosa is exposed to the irritants in the urine, which may rapidly trigger and exacerbate acute inflammation accompanied with bladder vascular hyperpermeability, edematous thickening, and infiltration of inflammatory cells, including neutrophils, into the submucosa, resulting increases in bladder weight. The H$_2$O$_2$-induced acute inflammation is evidenced by the rapid increase in mRNA expression level of inflammatory cytokines, such as TNF-$\alpha$, IL-1/$\beta$, and IL-6, in the bladder. In this H$_2$O$_2$-induced model, the bladder hyperactivity is induced by 1 day after the injection, followed by the acute bladder inflammation.

Subsequently, the H$_2$O$_2$-induced urothelial damage and hyperpermeability are recovered within several days. However, unlike other cystitis models, H$_2$O$_2$-induced
bladder hyperactivity lasts up to 7 days, accompanied with the vascular hyperpermeability and edematous thickening in the submucosa, as well as the increased mRNA expression of TNF-α and IL-1β. Taken together, it is suggested that the long-lasting bladder hyperactivity is induced by prolonged inflammatory responses triggered by H2O2-mediated acute injury to the bladder.

The inflammatory mediators such as inflammatory cytokines, chemokines, prostaglandins, and endogenous ROS, produced from infiltrated inflammatory cells could induce the hypersensitivity of the bladder sensory nerves, which can lead to the overactive bladder (30, 39, 40). The present findings are consistent with, at least in part, clinical features of IC patients. Numerous neutrophils are observed in the urothelial blood vessels and the submucosal connective tissue in biopsies from IC patients (1). Increased TNF-α, IL-1β, and IL-6 levels are observed in biopsies (41) and urine specimens taken from IC patients (42 – 46). Thus, the pathophysiology of H2O2-induced long-lasting inflammatory and overactive bladder is likely to reproduce a cycle of chronic inflammation in the bladder, which may represent common mechanisms for chronic cystitis, such as IC (47). It is noted that the H2O2 injection induced vascularization and hyperplasia in the submucosa, which may reflect an aspect of the pathophysiology of IC.

The present results showed that the therapeutic drugs clinically used for IC and overactive bladder, like oxybutynin, amitriptyline, and indomethacin, and a potent analgesic, morphine, attenuated the urinary frequency, but had no acute effect on the bladder inflammation in the H2O2-induced cystitis model. Anticholinergic drugs, such as oxybutynin, relieve urinary and bladder difficulties, including frequent urination and inability to control urination (urge incontinence), by decreasing muscle spasms of the bladder (48, 49). Tricyclic antidepressants, such as amitriptyline, may increase bladder capacity through β-adrenoceptors on the bladder and inhibition of the hypersensitivity of the bladder sensory nerves by blocking reuptake of noradrenaline and serotonin, as well as anticholinergic and antihistaminic effects (7, 50, 51). The anti-inflammatory effect of NSAIDs, such as indomethacin, helps to reduce the hypersensitivity of the bladder sensory nerves (52, 53), although the anti-inflammatory effect on the bladder inflammation, which was indicated by increased bladder weight and induction of TNF-α mRNA expression, was not observed in the present administration schedule. A potent opioid analgesic, morphine, may be used for severe bladder pain and referred pain in IC patients (34), while it suppresses urinary frequency through spinal and supraspinal mechanisms by acting on μ-opioid receptors (54, 55). Taken together, these results for drug sensitivity suggest that the H2O2-induced cystitis model is useful as hyperactive bladder model and is suitable to screen novel drugs for cystitis-induced urinary frequency. Further investigations will be needed to determine whether repeated administration of these drugs could affect the pathological processes of the H2O2-induced cystitis model.

A number of experimental animal models of cystitis are reported. For example, intravesical injection of acetic acid evokes acutely bladder hyperactivity measured by the continuous cystometrogram, 3 and 7 days after an intravesical injection of protamine sulphate in rats (18). However, although protamine sulphate promotes the acetic acid–evoked bladder hyperactivity, it does not induce spontaneous bladder hyperactivity. An intravesical co-injection of protamine sulphate with endotoxin lipopolysaccharide induces the inflammatory response in the epithelium, increased bladder permeability, and protease activity, which peaked at 4 h and disappeared within 24 h after the injection. In addition, repeated injection of protamine sulphate/lipopolysaccharide weekly for 5 weeks induces little or no inflammation in the bladder (19). Another report shows that repeated administration of low-dose CP induces “chronic” cystitis, but this model is in fact likely to be a “repetitive acute” cystitis model (56, 57). Following the repair of CP-induced acute typical hemorrhagic cystitis, delayed toxicity of CP, characterized by infiltration and transurothelial passage of inflammatory cells and frequent exfoliation of the urothelium, is induced 30 to 100 days after the CP treatment in female DBA/2 mice. However, the delayed toxicity of CP is mouse strain–specific and is not observed in C57BL/6 mice. In addition, it is unknown whether voiding frequency occurs in this model (58). According to the present findings, an intravesical H2O2–induced inflammatory and overactive bladder can be a relatively long-lasting cystitis model, rather than other existing cystitis models. However, further investigations will be needed to elucidate the mechanism of long-lasting inflammatory and overactive bladder.

In conclusion, the present study revealed that a single intravesical injection of H2O2 induces relatively long-lasting inflammatory and overactive bladder, which is responsive to the therapeutic drugs clinically used. Taken together, it is suggested that our mouse model of H2O2-induced cystitis is a novel chronic cystitis model reflecting an aspect of IC. The H2O2-induced chronic cystitis model may be a simple and useful tool in the pathological study and drug discovery for chronic cystitis such as IC.
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References

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