

Evaluation of urination in a rat cystitis model of induced by hydrogen peroxide

○ Hiromu Shimizu, Emi Morita, Saeko Yoshihara, Daichi Makabe, Ryoji Mizumachi, Takashi Tashiro, Seiichi Katayama, Naoyuki Hironaka, Katsuhide Nishi

Objective

Among various interstitial cystitis models in animals, few can be suitable for a long term evaluation. Although we have acetic acid-, hydrochloric acid-, and cyclophosphamide-induced models, the symptoms of pollakiuria recover within a week. Therefore, we tried to establish a cystitis model in which frequent urination symptoms continue for a long period of time using hydrogen peroxide (HP).

Female SD rats were used in the present study. First, we tested the induction time of HP and the maintenance period of symptoms. The model rats were prepared by injection of 3% HP into the bladder under isoflurane inhalation anesthesia. HP was stored in the bladder for 5, 15, or 30 min for separate groups. Uroflowmetry was measured at 1, 2, 3, 4, and 5 weeks after model preparation.

As the results, HP stored for 5 min shortened the urination interval, but individual differences were large. All rats died for 30 min condition. Therefore, we used 15 min thereafter. In this condition, the micturition interval was found to be shorten up to 2 weeks. Next, we tested the effects of amitriptyline (Grade B: Clinical Guideline for Interstitial Cystitis/Bladder Pain Syndrome) and celecoxib (Cox-2 inhibiting NSAID). Although amitriptyline improved the frequent urination symptoms, celecoxib did not show the improvement. As the conclusion, the HP-induced cystitis model is thought to be useful for evaluation of cystitis remedy because we could extend the observation period one week longer than the currently available models.

Summary in Japanese

排尿障害治療のため、各種の動物モデルおよび評価方法が用いられているが、長期間の評価が可能な膀胱炎モデルは少ない。我々が現有する酢酸、塩酸、シクロホスファミド誘発膀胱炎モデルでは、いずれも頻尿症状が1週間ほどで回復する。そこで今回、過酸化水素(H₂O₂)を用いて、長期に頻尿症状が継続する膀胱炎モデルの構築を行った。

雌性SDラットを用い、まずH₂O₂による膀胱炎の惹起時間の検討と病態モデルの維持期間を確認した。イソフルラン吸入麻酔下で3% H₂O₂を膀胱内に注入し、5分、15分、30分貯留する群を設け、その後1, 2, 3, 4, 5週でウロフロメトリー測定を行った。次にAmitriptyline, Celecoxibを用いて薬剤効果検証を行った。

膀胱内H₂O₂貯留5分では排尿間隔の短縮がみられたが個体差が大きく、30分では病態モデル作製3週後までに動物が死亡したため、以後の実験には15分を用いた。この条件でウロフロメトリー測定では病態モデル作製2週後まで排尿間隔の短縮が認められた。

薬効評価ではAmitriptyline(間質性膀胱炎・膀胱痛症候群診療ガイドラインで推奨グレードB)で頻尿症状の改善が認められたが、膀胱痛を抑えることで排尿機能を改善する可能性のあるCox-2阻害薬であるCelecoxibでは明らかな変化は見られなかった。

このモデルは現行モデルより1週間長く膀胱炎評価が可能であるため、膀胱炎治療薬の薬効評価に有用であると考えられた。

Materials and Methods

Experiment 1: Examination of model production conditions

Animals

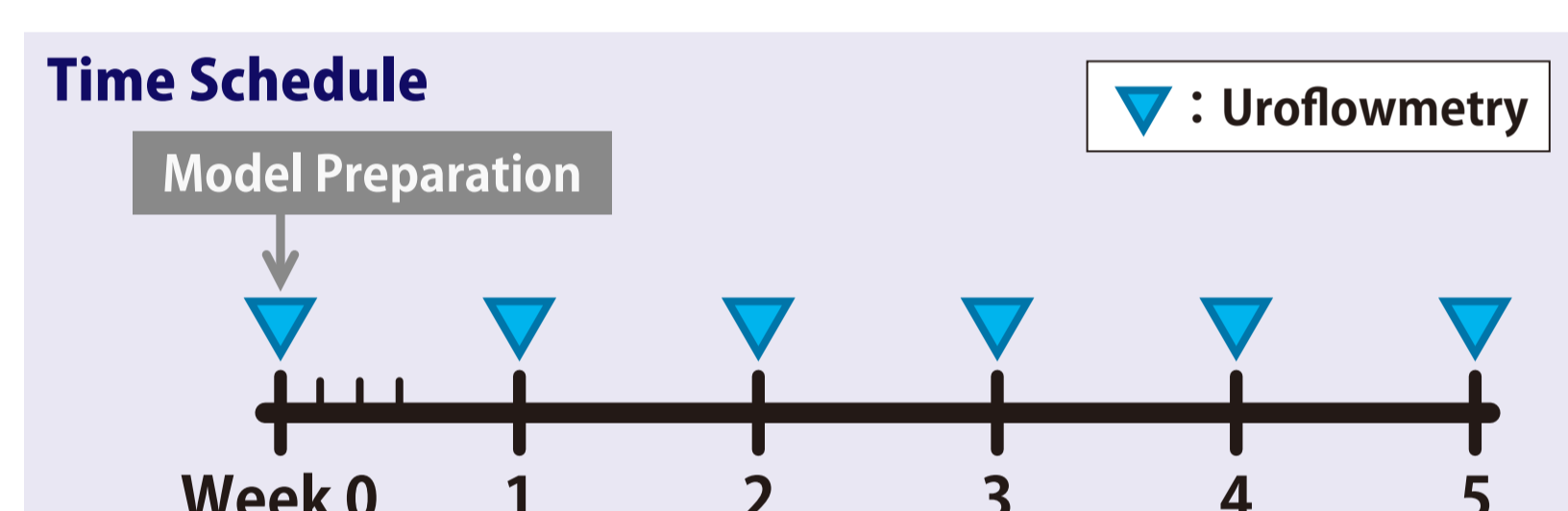
Female rat, Crl:CD(SD), 9-week-old at model preparation

Procedure

- Under 2% isoflurane anesthesia, a polyethylene tube was introduced into the bladder transurethraly and then the lower abdomen was pressed gently to withdraw urine.
- 300 μ L of 3% H₂O₂ solution dissolved in sterile saline was introduced into the bladder through the catheter.
- H₂O₂ in bladder was suctioned 5, 15, or 30 minutes after injection.
- Uroflowmetry was measured at 1, 2, 3, 4, and 5 weeks after model preparation.



| Test group | Dosing volume (mL/body) | 3% H ₂ O ₂ | N |
|------------|-------------------------|----------------------------------|---|
| Normal | - | - | 3 |
| 5 min | 0.3 | 5 min | 3 |
| 15 min | 0.3 | 15 min | 3 |
| 30 min | 0.3 | 30 min | 3 |



Experiment 2: Evaluation of drug efficacy in an H₂O₂-induced cystitis model

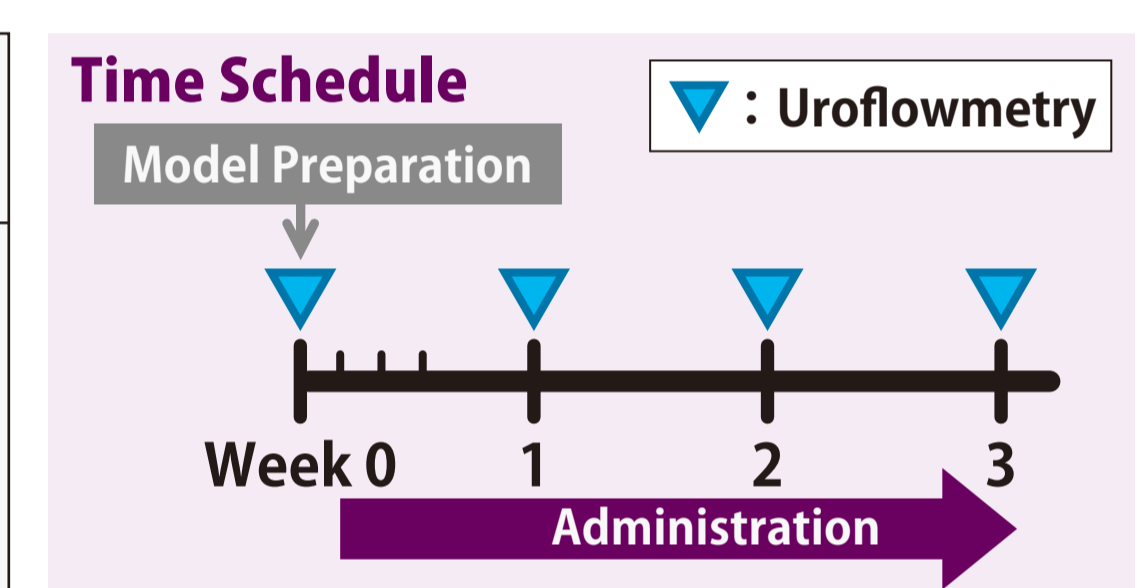
Animals

Female rat, Crl:CD(SD), 9-week-old at model preparation

Procedure

- The model was prepared with an intravesical retention time of 15 minutes under the conditions of Experiment 1.
- The dosing solutions were administrated orally once a day for 3 weeks from the day after model preparation.
- Uroflowmetry was measured at 1, 2, and 3 weeks after model preparation.

| Test group | Dosing solution | Dosing volume (mg/kg) | 3% H ₂ O ₂ | n |
|---------------|-----------------|-----------------------|----------------------------------|---|
| Normal | Saline | 0 | - | 5 |
| Control | Saline | 0 | + | 5 |
| Amitriptyline | Amitriptyline | 1 | + | 5 |
| Celecoxib | Celecoxib | 10 | + | 5 |



Results

Experiment 1

Fig. 1

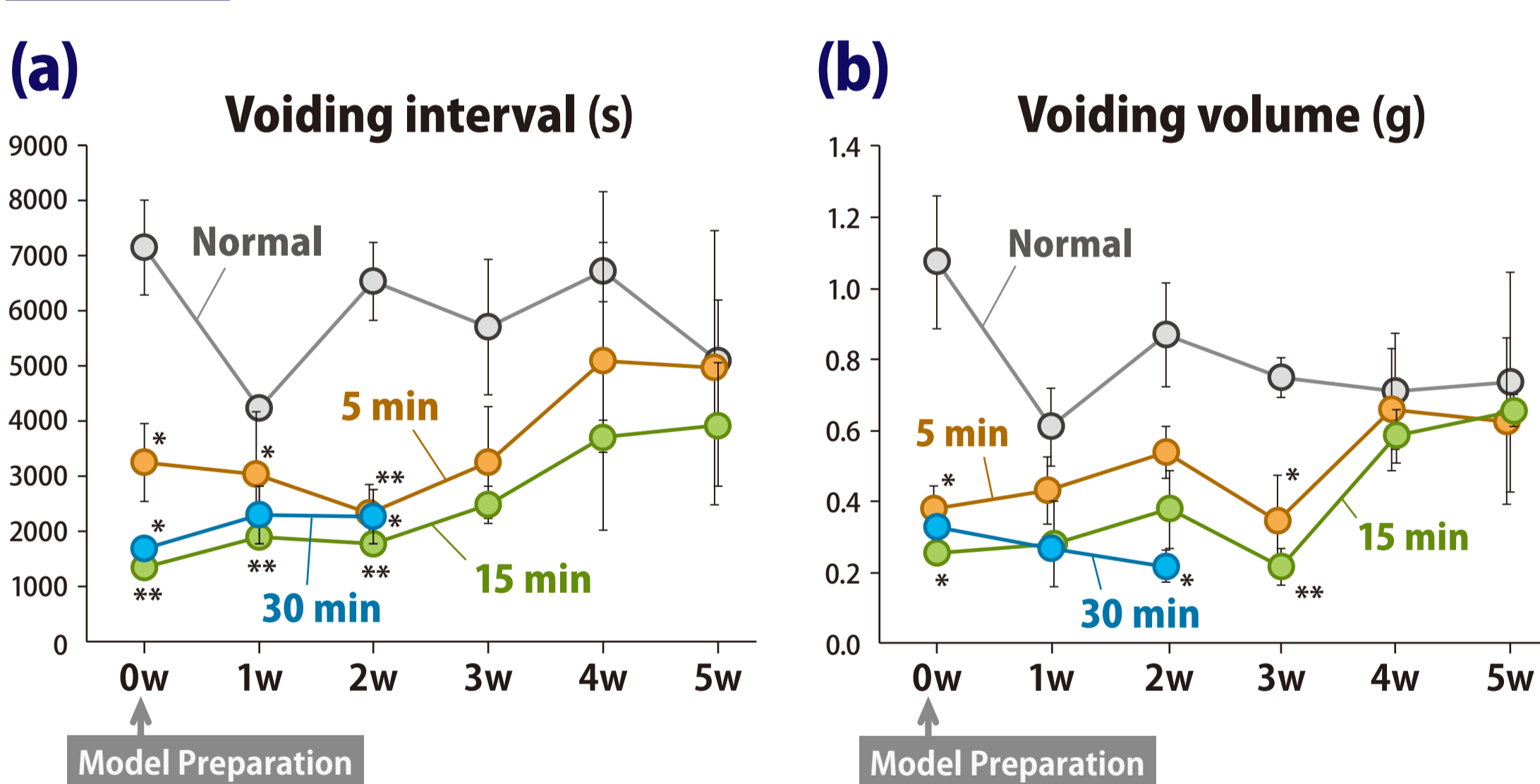
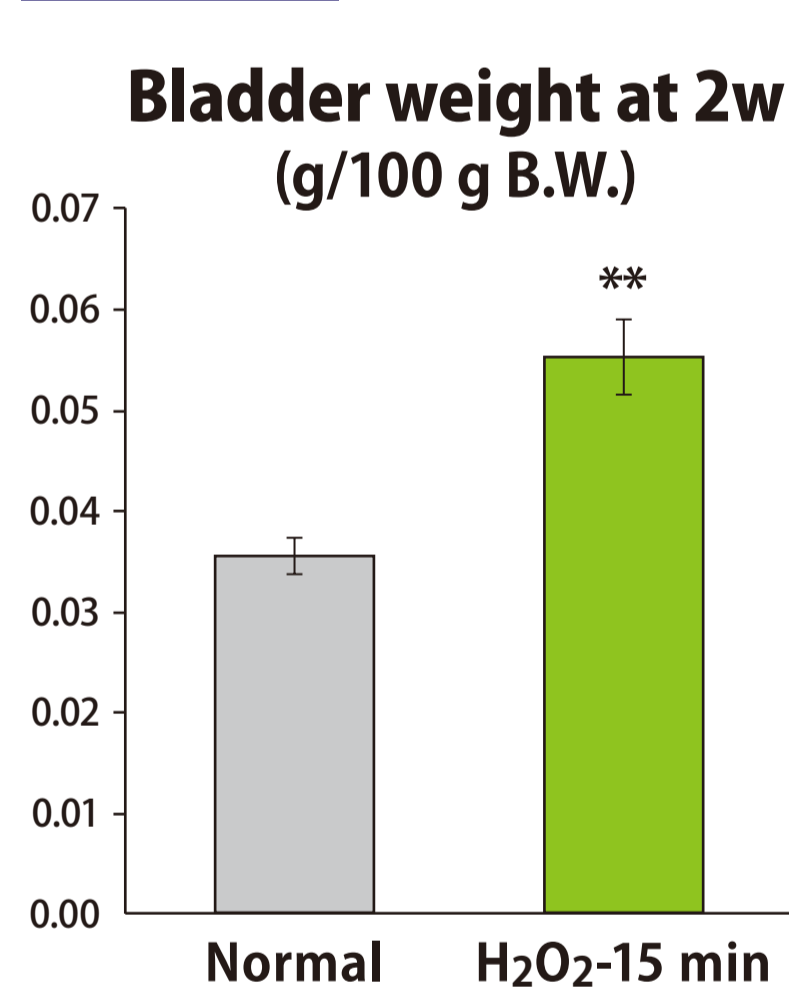
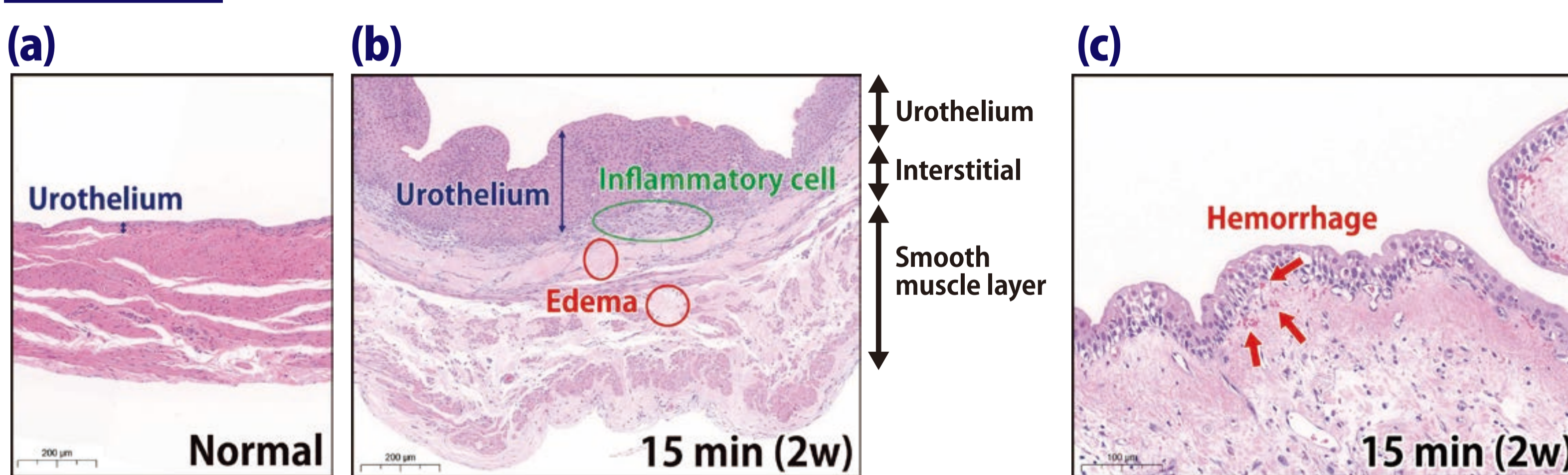


Fig. 2



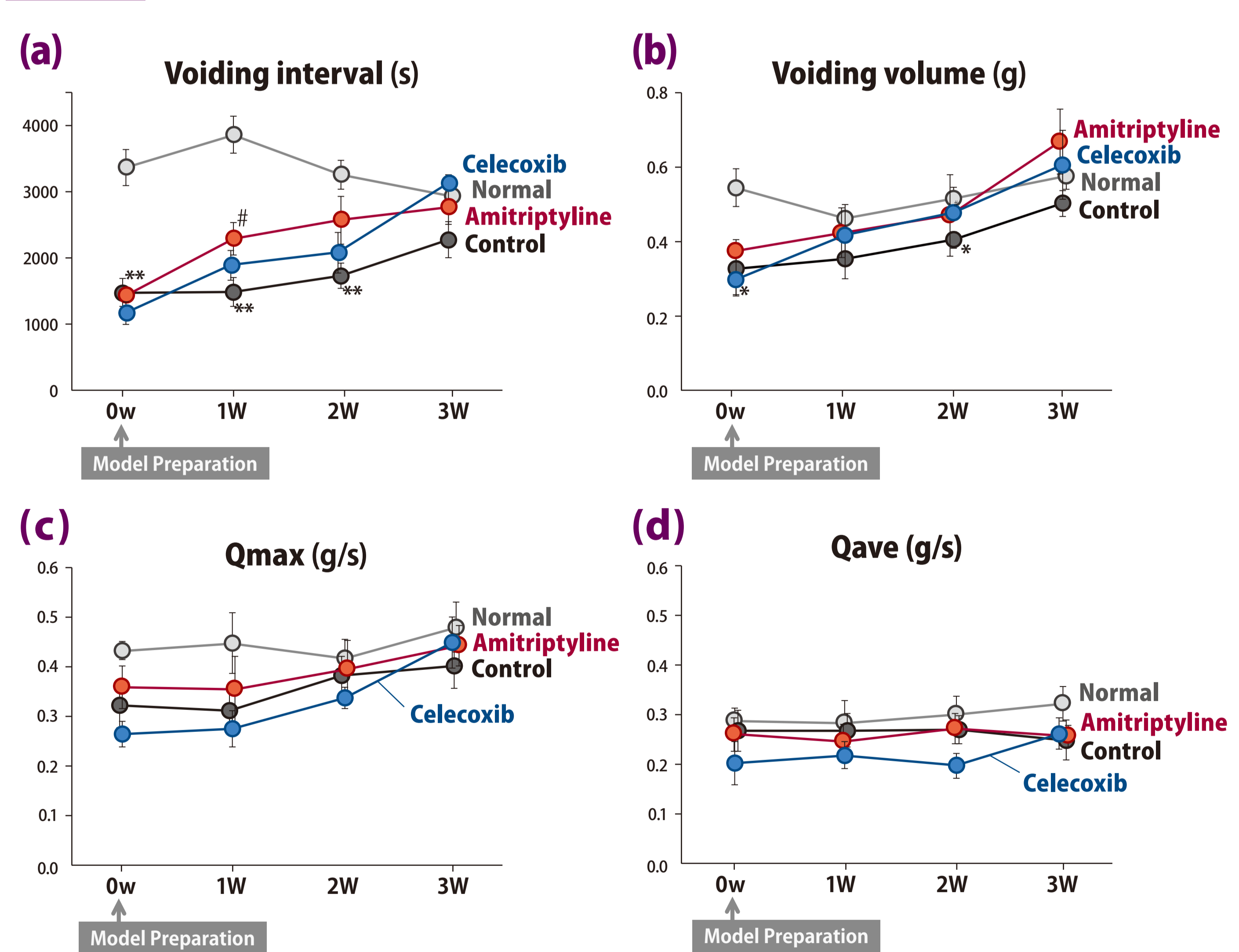
In 30 min group, all animals were dead by the time of the 3w measurement
Each value represents the mean \pm S.E. (n=3)
*: p<0.05, **: p<0.01; Significant difference from Normal group (Student's t-test)

Photo 1



Experiment 2

Fig. 3



Qmax: maximal urinary flow rate
Qave: average urinary flow rate
Each value represents the mean \pm S.E. (n=5)
*: p<0.05, **: p<0.01; Significant difference from Normal group (Student's t-test)
#: p<0.05; Significant difference from Control group (Student's t-test)

Discussion

Experiment 1

- When H₂O₂ was stored in the bladder for 5 min, the voiding interval was shortened, though there were large individual differences. H₂O₂ for 30 min was mortal [Fig. 1-(a)].
- In the H₂O₂-induced cystitis model, frequent urination lasted for up to 2 weeks and then gradually recovered [Fig. 1-(a),(b)].
- Uroepithelial hyperplasia, submucosal inflammatory cell infiltration and edema, and hemorrhage were observed in a 2-week model [Photo 1-(b),(c)].
- Bladder weight increased after 2 weeks [Fig. 2].

Since these findings similar to that found in humans, storage of H₂O₂ in the bladder for 15 min could be a valid model.

Experiment 2

- Amitriptyline improved frequent urination [Fig. 3-(a),(b)].
- No clear changes were observed by celecoxib [Fig. 3-(a),(d)].

The 3% H₂O₂-induced cystitis model showed longer-lasting symptoms. Moreover, the current histopathological findings were not obtained in our previous models. Since long-term evaluation of treatments could be observable, the current model is thought to be useful for evaluation of cystitis remedy.