〈Regular Article〉

Pathophysiological analysis of detrusor overactivity following partial bladder outlet obstruction

Hirofumi MORINAKA[1)], Shin OHIRA[1)], Shigenobu TONE[2)]
Sho KAKUMAE[1)], Tota NAKATSUKA[1)], Hiroyasu TAKASAKI[1)]
Keita HIRATA[1)], Shinjiro SHIMIZU[1)], Atsushi NAGAI[3)], Shinya UEHARA[1)]

1) Department of Urology, Kawasaki Medical School
2) Faculty of Science and Engineering, Tokyo Denki University
3) Director of Hospital, Kawasaki Medical School Hospital

ABSTRACT **Introduction:** Detrusor overactivity (DO) following partial bladder outlet obstruction (PBOO) is a common urological condition in humans, with 50-70% patients with PBOO complicated with DO. The pathological mechanisms of DO following PBOO are largely unknown, but inflammatory changes may play a key role. We hypothesized that inflammation is important in the earlier pathophysiological phase before overproduction of oxidative stress in DO following PBOO. Therefore, we investigated the relationships among bladder function, ischemia, oxidative stress and inflammation in DO following PBOO in PBOO model mice.

Materials and Methods: C57BL/6J male mice aged 10 to 15 weeks were used in the study. PBOO model mice were created surgically by ligation of the proximal urethra with 5-0 nylon suture under inhalation anesthesia. Sham-operated mice were used as controls. Pathophysiological changes in the bladder at 1, 3 and 5 weeks after creation of the PBOO model mice were compared with those in sham-operated mice using functional, histological, biochemical and immunohistochemical analyses.

Results: Functional analysis using a pressure flow study showed increased maximum detrusor pressure at 1 week and DO from 3 to 5 weeks after creation of the PBOO model. Histological analysis using hematoxylin-eosin and Masson-Trichrome staining showed greater invasion of inflammatory cells and fibrosis in PBOO model mice compared with sham-operated mice at 3 and 5 weeks. Inflammatory cells were mainly present in interstitial tissue, and fibrosis gradually infiltrated from interstitial tissue to the muscular layer. Ischemia analysis showed significantly increased HIF-1α in PBOO model mice at all time points. Oxidative stress analysis indicated significantly increased levels of ROS from 1 week and 8-OHdG from 3weeks in PBOO model mice. An inflammation-related proteome assay showed high levels of colony stimulating factor (CSF) family proteins at 1 week and IL-2, IL-3, IL-17A, IL-23, MMP-3, MMP-9 and periostin from

Corresponding author
Hirofumi Morinaka
Department of Urology, Kawasaki Medical School,
577 Matsushima, Kurashiki, 701-0192, Japan
Phone : 81 86 462 1111
Fax : 81 86 463 4747
E-mail: h.morinaka@med.kawasaki-m.ac.jp

3 to 5 weeks in PBOO model mice.

Conclusions: Oxidative stress and inflammatory changes showed contemporaneous increase in pathophysiology of detrusor overactivity following partial bladder outlet obstruction. Especially, CSF family and ROS changes are showed in the early stage, and might be a predict marker in the pathophysiology of DO following PBOO at the early stage.

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Key words：Detrusor overactivity, Partial bladder outlet obstruction, Bladder dysfunction, Pathophysiological analysis, Inflammation

INTRODUCTION

Partial bladder outlet obstruction (PBOO) is a common urological condition in humans with benign prostatic obstruction and lower urinary tract symptoms (LUTS), and is clinically defined as a high pressure and low flow micturition pattern in a urodynamic study[1, 2]. The bladder is continuously repeated the voiding and the storage phase, and is constantly under the ischemic state that a physiologically unavoidable condition[3, 4]. PBOO promotes ischemia in the bladder and induces various ischemic markers such as HIF-1 α[3, 4]. As a result, PBOO changes bladder structure and function, including detrusor hypertrophy, bladder wall thickness, elevated contractile pressure, detrusor instability, and leads to detrusor overactivity (DO)[5–7]. DO is also a common urological condition in humans, and is defined as urgency with or without urge incontinence, usually frequency and nocturia. About 50-70% of patients with PBOO are complicated with DO[8, 9].

Animal models of PBOO using ligation around the urethra have altered morphology and function, similar to human pathophysiology[10, 11]. Oxidative stress has been associated with pathological mechanisms of bladder damage following PBOO[10, 12–15], and PBOO can increase oxidative stress and affect bladder function via oxidative stress[15–18]. Oxidative stress is also associated with pathological mechanisms of DO following PBOO, and the pathological significance of oxidative stress in DO following PBOO is recognized in potential treatment strategies for bladder dysfunction[19]. However, monotherapy focused on oxidative stress may not be sufficient for DO following PBOO[19]. These findings suggest the need for intervention earlier in the pathophysiological process before overdevelopment of oxidative stress. Inflammatory changes have been suggested to play a key role in DO following PBOO[19, 20], but the association between oxidative stress and inflammatory changes after ischemic state in the bladder is still unclear. We hypothesized that inflammation is important in the earlier phase before development of excessive oxidative stress. In this study, we investigated the relationships among inflammation, oxidative stress and bladder function in DO following PBOO in PBOO model mice.

MATERIALS AND METHODS

Research approval and model preparation

The study was approved by the animal research committee of Kawasaki Medical School, Kurashiki, Japan (approval numbers 19-048 and 21-052) and was conducted according to the guidelines for the care and use of laboratory animals of Kawasaki Medical School. C57BL/6J male mice (Jackson Laboratory, Bar Harbor, ME, USA) aged 10 to 15 weeks were used in the study, and were divided into PBOO model and sham-operated groups. A well-described PBOO mouse model[21–23] was created using the following method. The bladder was

exposed via a midline incision and PBOO model mice were created by ligation of the proximal urethra using 5-0 nylon suture under inhalation anesthesia with sevoflurane (Pfizer Japan Inc., Tokyo, Japan). The urethral diameter was adjusted to 0.6 mm. Sham-operated mice underwent the same procedure, except for ligation. Non-operated C57BL/6J mice under same condition were set as control mice. Pathophysiological changes in the bladder at 1, 3 and 5 weeks after creation of PBOO model mice were compared with those in sham-operated mice. Bladders used in each analysis and observation period were not used in other experiments to obtain accurate measurements.

Functional analysis

Functional changes in the bladder were investigated using a pressure flow study (PFS) at 1, 3 and 5 weeks after creation of PBOO model mice and sham-operated mice. The dome of the bladder was punctured using a 26G needle (Terumo, Tokyo, Japan) and drained. An extension tube with 3 stopcocks (Terumo) was connected to the needle and normal saline was injected into the bladder at 100 μL/min using a syringe pump (KDS200, Brain Science Idea, Osaka, Japan) under inhalation anesthesia with sevoflurane. Intravesical pressure was measured using a Bio Research System (SEN-6102M, Nihon Kohden, Tokyo, Japan) and recorded using a Lab Chart Reader (Bio Research Center, Nagoya, Japan). Maximum detrusor pressure (maximum pressure recorded at the first urination), bladder capacity (calculated as the first urination time multiplied by 100 μL/min) and detrusor overactivity (bladder contraction independent of urination and analyzed in repeated urinations with continuous injection of normal saline at 100 μL/min) were examined.

Histological analysis

Bladders were removed under inhalation anesthesia with sevoflurane at 1, 3 and 5 weeks after model creation. Resected bladder tissues were fixed in 10% formalin neutral buffer solution (Wako Pure Chemical, Osaka, Japan). Fixed tissues were dehydrated in an ethanol series and finally in absolute ethanol. Dehydrated tissues were embedded in paraffin wax, cut to a thickness of 5 μm, mounted on slides, and dried for 45 min at 45℃. Tissue slides were dewaxed and hydrated through ethanol-graded solutions to water. Histological analysis using hematoxylin-eosin (HE) and Masson-Trichrome (MT) staining was performed using an Olympus BX-53 microscope (Olympus, Tokyo, Japan).

Biochemical analysis

Biochemical changes in the bladder were investigated using a similar procedure to that described above for bladder tissue. Tissue was homogenized with Cell Lysis Buffer and PMSF, and tissue fragments were removed after centrifugation (15000 × g, 5 min, 5℃). Ischemia and oxidative stress in the bladder were evaluated using enzyme-linked immuno-sorbent assays (ELISAs) for hypoxia inducible factor (HIF)-1 α (Cosmo Bio, Tokyo, Japan), reactive oxygen species (ROS) (R&D Systems, Minneapolis, MN, USA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) (Japan Institute for the Control of Aging, Shizuoka, Japan). Inflammatory changes in the bladder were investigated using an inflammation-related proteome assay (Proteome Profiler Mouse XL Cytokine Array, R&D Systems). The protein concentration of each sample was 300 μg/mL. The pixel density (PD) was calculated using an image analyzer (Image Quant LAS4000 mini, GE Healthcare Japan, Tokyo, Japan) and image analysis software (Image Quant TL, GE Healthcare Japan). The calculated PD was corrected using endogenous controls, with negative and positive controls set as PD 0 and 100, respectively. As the initial extraction of inflammatory markers that indicates of pathophysiology in DO following PBOO, we

extracted 10 types of cytokines and chemokines that had a ratio of 5 times or more between PBOO model and sham-operated mice at any observation point. Furthermore, the extracted 10 candidates were subjected to comparative analysis between PBOO model and sham-operated mice after calculating the amount of change at each observation periods.

Immunohistochemical analysis

Immunohistochemical analysis was performed using immunofluorescent staining (ImmPRESS™-AP Reagent, Vector Laboratories, CA, USA). Blocking was performed for 10 min at room temperature with BLOXALL™ blocking solution (Vector Laboratories) after de-waxing and hydration. Based on the results of biochemical analysis, primary antibodies against indicators of ischemia: HIF-1 α, oxidative stress: ROS, neutrophils: G-CSF and F4/80, T-lymphocytes: G-CSF and IL-2, monocytes and macrophages: M-CSF and IL-17 were used, with all diluted in 2.5% normal horse serum (Vector Laboratories): anti-HIF-1 α antibody (#3434, Cell Signaling Technology, CA, USA; diluted 3000-fold); anti-ROS antibody (sc-28974, Santa Cruz Biotechnology, Santa Cruz, CA, USA; diluted 100-fold); G-CSF (bs-1023R, Bioss Inc., MA, USA; diluted 200-fold); M-CSF (bs-4910R, Bioss Inc., MA, USA; diluted 1100-fold); F4/80 (#70076, Cell Signaling Technology, CA, USA; diluted 125-fold); IL-2 (ab180780, Abcam plc, Cambridge, UK; diluted 100-fold); and IL-17 (ab79056, Abcam plc, Cambridge, UK; diluted 200-fold). Rabbit IgG Control Antibody (Vector Laboratories; diluted 2000-fold) was used as negative control (NC). The primary antibody reaction was performed overnight at 4℃. The secondary antibody reaction was performed using ImmPRESS™-AP Reagent Anti-Rabbit IgG Polymer Kit (Vector Laboratories) for 30 min at room temperature. The immune substrates were ImmPACT™ Vector Red Alkaline Phosphatase (Vector Laboratories). Counterstain was performed using 4', 6-diamidino-2-phelylindole (DAPI, Vector laboratories). After encapsulation using VectaMount™ Permanent Mounting Medium (Vector laboratories), immunohistochemical analysis was examined using an Olympus BX-53 microscope (Olympus, Tokyo, Japan).

Statistical analysis

Excel Statistics (Microsoft, Redmond, WA, USA) was used for statistical analysis. The amount of change at over time in PBOO model and sham-operated mice were shown as the value obtained by subtracting the standard value in control mice ($n = 4$) from the measured value in PBOO model or sham-operated mice. Differences in amount of change between PBOO model and sham-operated mice were analyzed by Welch test, with $p < 0.05$ considered to indicate a significant difference. The PD range was defined as 0 (negative control) to 100 (positive control) and expressed as a PD ratio (PD for PBOO model mice / PD for sham-operated mice).

RESULTS

Functional changes in PBOO model mice

The PFS showed a significantly higher the change amount of maximum detrusor pressure and bladder capacity in PBOO model mice compared with sham-operated mice at 1, 3 and 5 weeks after model creation, and the bladder capacity in PBOO model mice compared with sham-operated mice at 5 weeks after model creation (Table 1). The increased maximum detrusor pressure and bladder capacity indicates exacerbation of bladder outlet obstruction and hyperextension of the bladder in PBOO model mice. These results indicated that functional changes as an increase of the maximum detrusor pressure appeared in the early stages, and organic changes as an increase of the bladder capacity appeared at over time. Detrusor overactivity at 3 and 5 weeks

Table 1. PFS: maximum detrusor pressure and bladder capacity analysis at over time

Change amount of maximum detrusor pressure (mmHg)		Standard value	mean	SE	p value
Week1	Sham	14.25	+0.75	2.65	0.0063
	Model		+16.25	1.32	
Week3	Sham		+2.50	2.93	0.0043
	Model		+19.25	1.71	
Week5	Sham		-0.75	2.02	0.0070
	Model		+15.50	3.09	
Change amount of bladder capacity (μL)		Standard value	mean	SE	p value
Week1	Sham	219.75	+8.75	19.96	0.1544
	Model		+49.00	13.33	
Week3	Sham		+17.50	25.13	0.1193
	Model		+73.00	15.57	
Week5	Sham		+27.00	18.02	0.0022
	Model		+149.00	15.72	

The change amount of maximum detrusor pressure was significantly higher in PBOO model mice than in sham-operated mice at 3 and 5 weeks. The increased maximum detrusor pressure indicates exacerbation of bladder outlet obstruction in PBOO model mice. Data are shown as mean and standard error (SE). The amount of changes were shown as the value obtained by subtracting the standard value in control mice (n = 4) from the measured value. Differences in amount of change between PBOO model and sham-operated mice were analyzed by Welch test. n = 4 for each week.

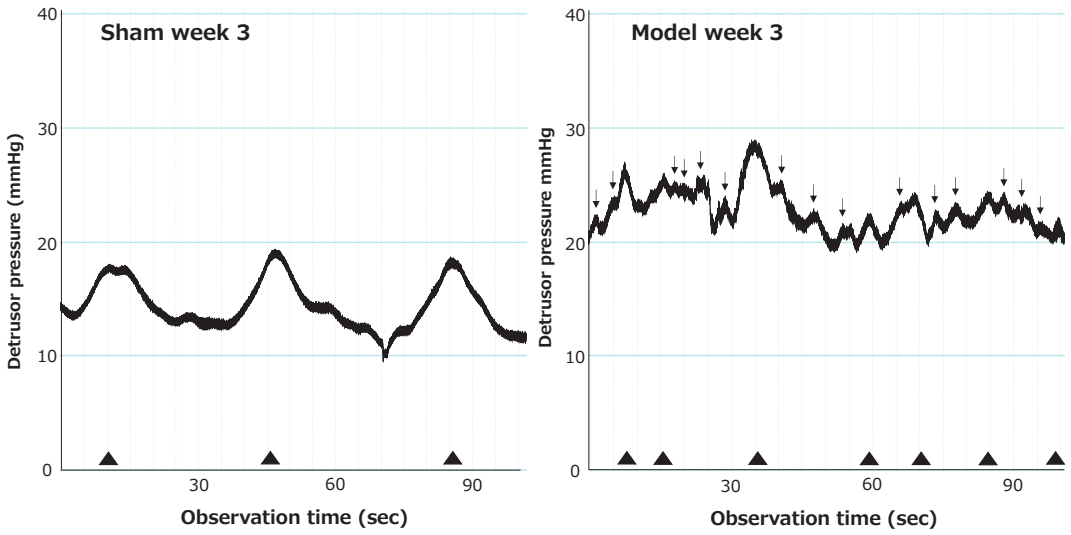

Fig. 1. PFS: presence of detrusor overactivity analysis
Detrusor overactivity was found in PBOO model mice compared with sham-operated mice. This overactivity indicates a compensation function due to bladder outlet obstruction. arrowhead: urination, arrow: detrusor overactivity.

was also present in PBOO model mice (Fig. 1: only 3 weeks is shown). This indicates a compensation function due to bladder outlet obstruction.

Histological changes in PBOO model mice

HE staining showed greater invasion of inflammatory cells in PBOO model mice compared with sham-operated mice at 3 and 5 weeks (Fig.

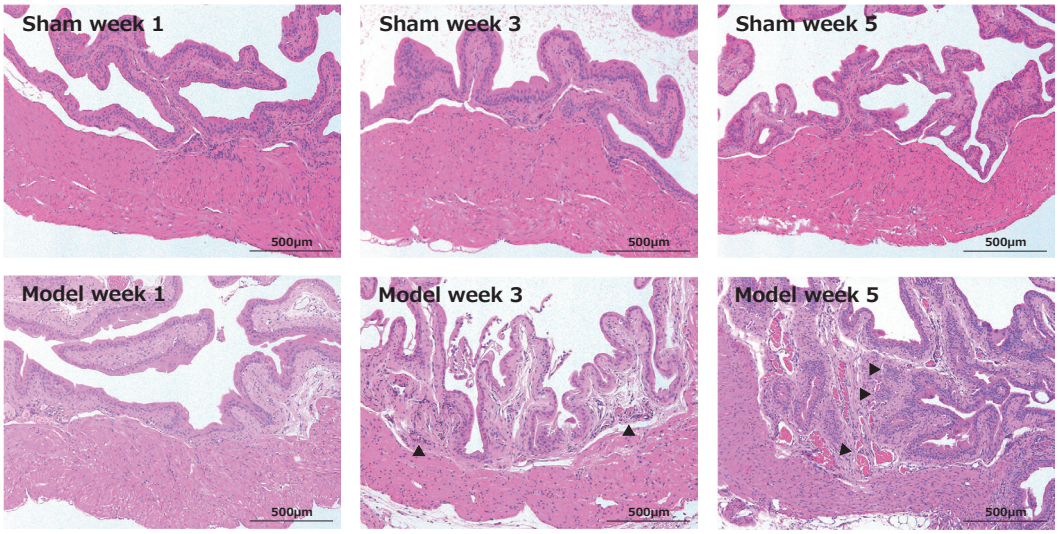

Fig. 2. Histological analysis: HE staining in the bladder at over time
HE staining showed greater invasion of inflammatory cells (arrowhead) in PBOO model mice than in sham-operated mice at 3 and 5 weeks. Inflammatory cells were mainly present in interstitial tissue. Scale bar: 500 μm. Magnification: 100×. n = 4 for each week.

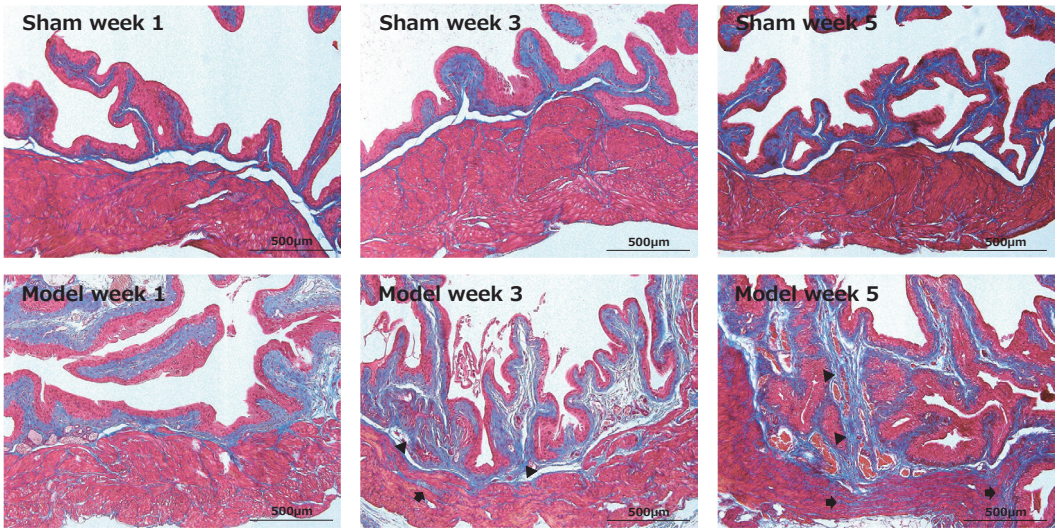

Fig. 3. Histological analysis: MT staining in the bladder at over time
MT staining showed more tissue fibrosis (arrowhead) in PBOO model mice than in sham-operated mice at 3 and 5 weeks, with fibrosis mainly in interstitial tissue. Fibrosis gradually infiltrated into the muscular layer (arrow). Scale bar: 500 μm. Magnification: 100×. n = 4 for each week.

2). Inflammatory cells were mainly present in interstitial tissue. MT staining showed more tissue fibrosis in PBOO model mice at 3 and 5 weeks, with fibrosis mainly in interstitial tissue (Fig. 3). Fibrosis gradually infiltrated into the muscular layer.

Ischemic changes in PBOO model mice

The change amount of HIF-1 α assay showed significantly greater ischemia changes in PBOO model mice compared with sham-operated mice at all time points after model creation (Table

2). Ischemic changes in PBOO mice tended to gradually decrease, but quantitative assay showed significantly higher ischemic changes in PBOO model mice compared with sham-operated mice at all observation periods. The decreased expression of ischemic changes may involve tissue destruction and fibrosis in histological analysis.

Oxidative stress changes in PBOO model mice

The change amount of ROS assay showed significantly higher value in PBOO model mice compared with sham-operated mice at all observation periods (Table 3). The change amount of 8-OHdG assay showed significantly higher value in PBOO model mice compared with sham-operated mice at 3 and 5 weeks (Table 3). These results indicated that ROS assay may reflect more earlier stage of pathophysiology than 8-OHdG assay.

Inflammatory changes in PBOO model mice

The 10 types of cytokines and chemokines were extracted from the initial extraction that had a ratio of 5 times or more between PBOO model and sham-operated mice at any observation periods (Table 4).

Table 2. Biochemical analysis: HIF-1 α assay (ELISA) in the bladder at over time

Change amount of HIF-1 (pg/mL)		Standard value	mean	SE	p value
Week1	Sham	199.08	+77.98	56.95	0.0002
	Model		+1108.92	120.23	
Week3	Sham		+97.52	50.55	0.0003
	Model		+1015.34	107.90	
Week5	Sham		+72.64	45.36	0.0003
	Model		895.38	98.44	

HIF-1 α was significantly higher in PBOO model mice compared with sham-operated mice at all time points. Data are shown as mean and standard error (SE). The amount of changes were shown as the value obtained by subtracting the standard value in control mice (n = 4) from the measured value. Differences in amount of change between PBOO model and sham-operated mice were analyzed by Welch test. n = 4 for each week.

Table 3. Biochemical analysis: ROS and 8-OHdG assay (ELISA) in the bladder at over time

Change amount of ROS (μg/mL)		Standard value	mean	SE	p value
Week1	Sham	218.52	+0.75	2.65	0.0002
	Model		+16.25	1.32	
Week3	Sham		+2.50	2.93	0.0003
	Model		+19.25	1.71	
Week5	Sham		-0.75	2.02	0.0003
	Model		+15.50	3.09	
Change amount of 8-OHdG (ng/mL)		Standard value	mean	SE	p value
Week1	Sham	31.41	+0.28	7.37	0.2489
	Model		+14.15	8.38	
Week3	Sham		-1.73	11.11	0.0003
	Model		+91.03	10.88	
Week5	Sham		+0.80	8.39	0.0001
	Model		+94.27	10.88	

ROS levels were significantly higher in PBOO model mice than in sham-operated mice at all observation periods. 8-OHdG was significantly higher in PBOO model mice than in sham-operated mice at 3 and 5 weeks. Data are shown as mean with standard error (SE). The amount of changes were shown as the value obtained by subtracting the standard value in control mice (n = 4) from the measured value. Differences in amount of change between PBOO model and sham-operated mice were analyzed by Welch test. n = 4 for each week.

Table 4. Inflammation-related proteome assay in the bladder at over time

Change amount of GM-CSF (Pixel density)		Standard value	mean	SE	p value
Week1	Sham	0.78	+0.01	0.07	0.0072
	Model		+7.03	1.07	
Week3	Sham		+0.01	0.07	0.0024
	Model		+4.57	0.47	
Week5	Sham		+0.01	0.07	0.0037
	Model		+4.35	0.52	
Change amount of G-CSF (Pixel density)		Standard value	mean	SE	p value
Week1	Sham	1.09	+0.08	0.08	0.0029
	Model		+9.09	1.00	
Week3	Sham		+0.08	0.08	0.0024
	Model		+5.81	0.59	
Week5	Sham		+0.08	0.08	0.0021
	Model		+7.68	0.75	
Change amount of M-CSF (Pixel density)		Standard value	mean	SE	p value
Week1	Sham	0.89	+0.01	0.07	0.0107
	Model		+6.58	1.15	
Week3	Sham		+0.01	0.07	0.0163
	Model		+2.75	0.56	
Week5	Sham		+0.01	0.07	0.0093
	Model		+4.12	0.68	
Change amount of IL-2 (Pixel density)		Standard value	mean	SE	p value
Week1	Sham	0.51	+0.01	0.12	0.0044
	Model		+3.76	0.47	
Week3	Sham		+0.01	0.12	0.0145
	Model		+5.51	1.07	
Week5	Sham		+0.01	0.12	0.0038
	Model		+5.18	0.62	
Change amount of IL-3 (Pixel density)		Standard value	mean	SE	p value
Week1	Sham	0.49	+0.01	0.09	0.0033
	Model		+2.88	0.31	
Week3	Sham		+0.01	0.09	0.0156
	Model		+3.97	0.79	
Week5	Sham		+0.01	0.09	0.0039
	Model		+4.25	0.52	
Change amount of IL-17 (Pixel density)		Standard value	mean	SE	p value
Week1	Sham	0.33	+0.01	0.09	0.0235
	Model		+1.96	0.45	
Week3	Sham		+0.01	0.09	0.0146
	Model		+3.10	0.60	
Week5	Sham		+0.01	0.09	0.0142
	Model		+3.41	0.66	
Change amount of IL-23 (Pixel density)		Standard value	mean	SE	p value
Week1	Sham	0.40	+0.01	0.08	0.0374
	Model		+1.88	0.52	
Week3	Sham		+0.01	0.08	0.0138
	Model		+3.34	0.64	
Week5	Sham		+0.01	0.08	0.0108
	Model		+3.07	0.53	

Change amount of MMP-3 (Pixel density)		Standard value	mean	SE	p value
Week1	Sham	0.46	+0.01	0.07	0.0385
	Model		+2.25	0.63	
Week3	Sham		+0.01	0.07	0.0003
	Model		+6.47	0.72	
Week5	Sham		+0.01	0.07	0.0067
	Model		+7.08	1.05	
Change amount of MMP-9 (Pixel density)		Standard value	mean	SE	p value
Week1	Sham	0.33	+0.01	0.07	0.0492
	Model		+1.48	0.46	
Week3	Sham		+0.01	0.07	0.0032
	Model		+3.88	0.44	
Week5	Sham		+0.01	0.07	0.0045
	Model		+4.03	0.52	
Change amount of Periostin (Pixel density)		Standard value	mean	SE	p value
Week1	Sham	0.36	+0.01	0.07	0.0005
	Model		+0.77	0.09	
Week3	Sham		+0.01	0.07	0.0024
	Model		+5.08	0.52	
Week5	Sham		+0.01	0.07	0.0012
	Model		+5.11	0.42	

The 10 types of cytokines and chemokines were extracted from the initial extraction that had a ratio of 5 times or more between PBOO model and sham-operated mice at any observation periods. The amount of change in extracted 10 candidates showed significant increases in PBOO model mice compared with sham-operated mice at all observation periods. The quantitative assay also showed higher levels of CSF family proteins at 1 week after creation of the PBOO model compared with sham-operated mice, and higher levels of IL-2, IL-3, IL-17A, IL-23, MMP-3, MMP-9 and periostin in the PBOO model compared with sham-operated mice at 3 and 5 weeks. Data are shown as mean with standard error (SE). The amount of changes were shown as the value obtained by subtracting the standard value in control mice (n = 4) from the measured value. Differences in amount of change between PBOO model and sham-operated mice were analyzed by Welch test. n = 4 for each week.

The amount of change in extracted 10 candidates showed significant increases in PBOO model mice compared with sham-operated mice at all time points (Table 4). PBOO model mice had higher levels of colony stimulating factor (CSF) family proteins (granulocyte macrophage (GM)-CSF, granulocyte (G)-CSF and macrophage (M)-CSF) at 1 week and tended to gradually decrease. But CSF family assay showed significantly higher values in PBOO model mice compared with sham-operated mice at all observation periods (Table 4). Furthermore, PBOO model mice had higher levels IL-2, IL-3, IL-17A, IL-23, matrix metalloproteinase (MMP)-3, MMP-9 and periostin at 3 and 5 weeks (Table 4). But, IL-2, IL-3, IL-17A, IL-23, MMP-3, MMP-9 and periostin assay also showed significantly higher values in PBOO model mice compared with sham-operated mice at all observation periods (Table 4). These results indicated that CSF family assay may reflect more earlier stage of pathophysiology than IL-2, IL-3, IL-17A, IL-23, MMP-3, MMP-9 and periostin assay.

Localization of pathological markers in PBOO model mice

Immunofluorescent staining showed increased expression of HIF-1α, ROS, G-CSF, M-CSF, F4/80, IL2 and IL-17 in PBOO model mice at 1 week (Fig. 4) and 3 weeks (Fig. 6) compared with sham-operated mice at 1 week (Fig. 5) and 3 weeks (Fig. 7). Expression of HIF-1α in PBOO mice were mainly showed in intravascular and

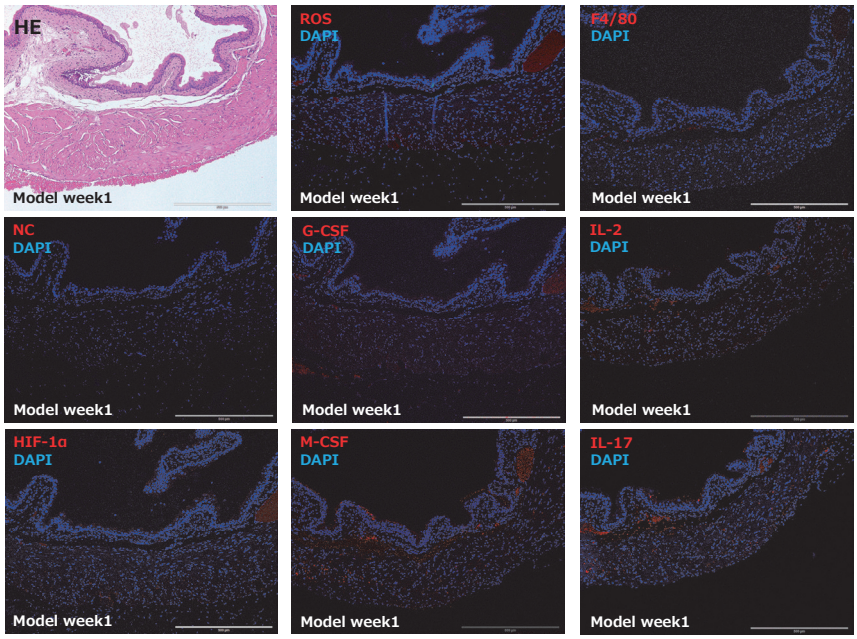

Fig. 4. Immunohistochemical analysis: immunofluorescent staining in PBOO model mice at 1 week
Immunostaining showed expression of HIF-1 α, ROS, G-CSF, M-CSF, F4/80, IL2 and IL-17 in PBOO model mice at 1 week. Expression of inflammatory cytokines that was mainly composed of T-lymphocytes related CSF (G-CSF) and monocytes and macrophages related CSF (M-CSF) in PBOO mice were observed at all layers in the bladder. HIF-1 α: indicator of ischemia. ROS: indicator of oxidative stress. G-CSF and F4/80: indicators of neutrophil. G-CSF and IL-2: indicators of T-lymphocyte. M-CSF and IL-17: indicator of monocyte and macrophage. NC (negative control): Rabbit IgG (Control Antibody). Scale bar: 500 μm. Magnification: LPF 100×. n = 4 for each week.

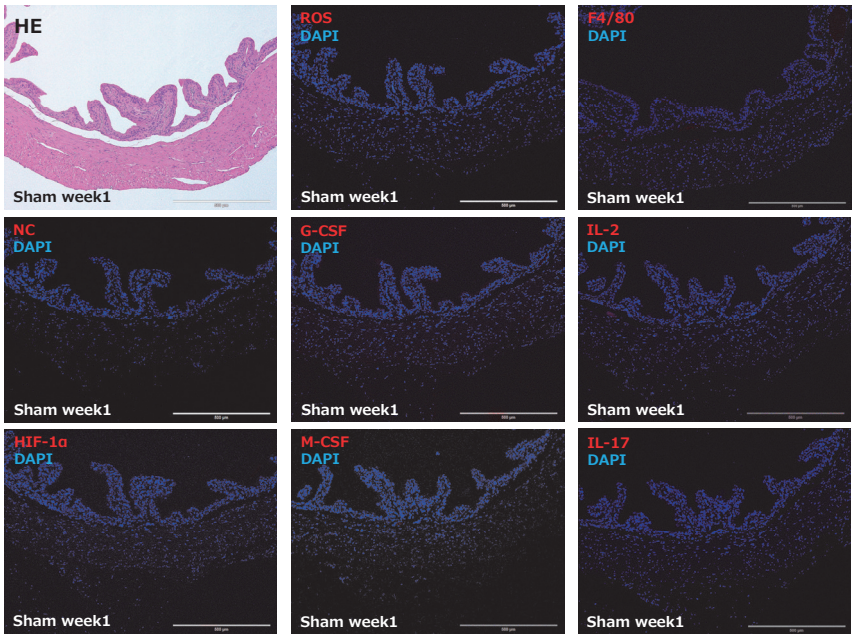

Fig. 5. Immunohistochemical analysis: immunofluorescent staining in sham-operated mice at 1 week
Immunostaining showed less expression of HIF-1 α, ROS, G-CSF, M-CSF, F4/80, IL2 and IL-17 in sham-operated mice at 1 week. HIF-1 α: indicator of ischemia. ROS: indicator of oxidative stress. G-CSF and F4/80: indicators of neutrophil. G-CSF and IL-2: indicators of T-lymphocyte. M-CSF and IL-17: indicator of monocyte and macrophage.
NC (negative control): Rabbit IgG (Control Antibody). Scale bar: 500 μm. Magnification: LPF 100×. n = 4 for each week.

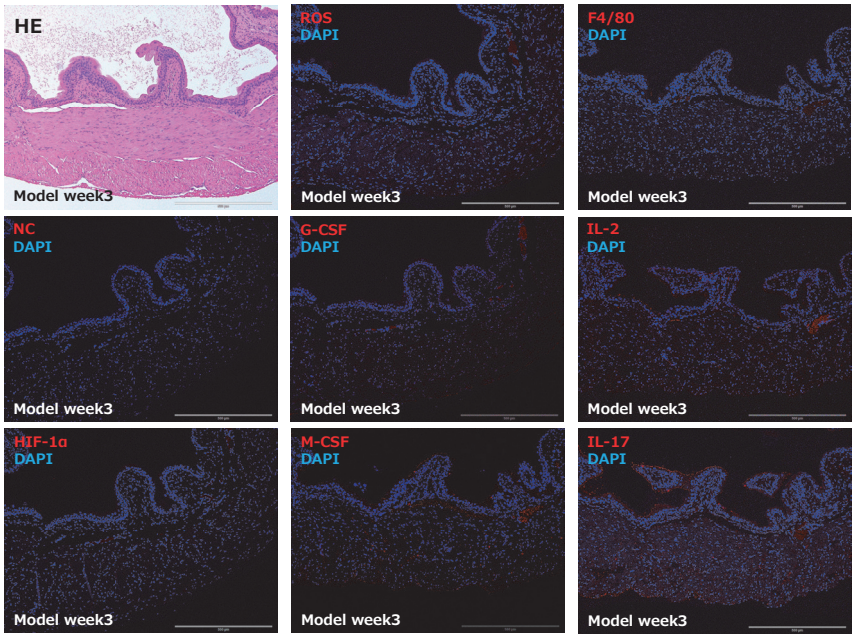

Fig. 6. Immunohistochemical analysis: immunofluorescent staining in PBOO model mice at 3 weeks
Immunostaining showed expression of HIF-1 α, ROS, G-CSF, M-CSF, F4/80, IL2 and IL-17 in PBOO model mice at 3 weeks. Expression of inflammatory cytokines that was mainly composed of T-lymphocytes related cytokines (IL-2) and monocytes and macrophages related cytokines (IL-17) in PBOO mice were observed at all layers. Expression of IL-2 and IL-17 were gradually expanded at 3 weeks compared with that of 1 week. HIF-1 α: indicator of ischemia. ROS: indicator of oxidative stress. G-CSF and F4/80: indicators of neutrophil. G-CSF and IL-2: indicators of T-lymphocyte. M-CSF and IL-17: indicator of monocyte and macrophage.NC (negative control): Rabbit IgG (Control Antibody). Scale bar: 500 μm. Magnification: LPF 100×. n = 4 for each week.

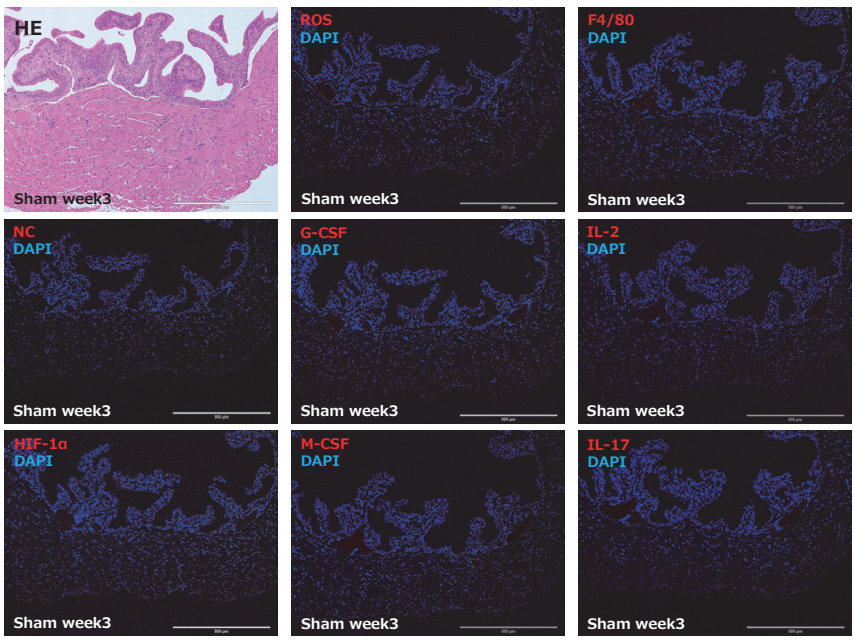

Fig. 7. Immunohistochemical analysis: immunofluorescent staining in sham-operated mice at 3 weeks
Immunostaining showed less expression of HIF-1 α, ROS, G-CSF, M-CSF, F4/80, IL2 and IL-17 in sham-operated mice at 3 weeks following at 1 week. HIF-1 α: indicator of ischemia. ROS: indicator of oxidative stress. G-CSF and F4/80: indicators of neutrophil. G-CSF and IL-2: indicators of T-lymphocyte. M-CSF and IL-17: indicator of monocyte and macrophage. NC (negative control): Rabbit IgG (Control Antibody). Scale bar: 500 μm. Magnification: LPF 100×. n = 4 for each week.

perivascular tissues at 1 week (Fig. 4) and 3 weeks (Fig. 6). Expression of ROS in PBOO mice were showed in intravascular, perivascular, interstitial and muscular tissues at 1 week, and gradually expanded at 3 weeks (Fig. 6). Expression of inflammatory cytokines that was mainly composed of T-lymphocytes related CSF (G-CSF) and monocytes and macrophages related CSF (M-CSF) in PBOO mice were showed at all layers in the bladder from 1 week (Fig. 4). Expression of T-lymphocytes related cytokine (IL-2) and monocytes and macrophages related cytokine (IL-17) were mainly showed in all layers in the bladder at 3 weeks (Fig. 6).

DISCUSSION

This study showed that highly expression of CFS family and ROS, and functional changes at the early stage, and highly expression of CFS-, muscle layer collapse- and fibrosis-related cytokine and 8-OHdG, organic changes at the following stage in the pathophysiology of DO following PBOO. The results suggested the possibility of contemporaneous expression of inflammatory changes and oxidative stress in the early stage. Therefore, if we can monitor the change of CFS family and ROS in PBOO, we may predict the pathophysiology of DO following PBOO at the early stage.

PBOO induces ischemic-reperfusion injury in the bladder and high-pressure urination[1]. Subsequently, this bladder injury leads to smooth muscle disorder and bladder dysfunction (DO) via overproduction of oxidative stress and inflammation[1]. However, DO following PBOO has a complex pathophysiology that remains to be elucidated. Furthermore, studies using human material are limited by legal, ethical and moral concerns. Therefore, animal models in pig, dog, rabbit, guinea pig, rat and mouse have been used for research on LUTS[24]. These models can be roughly divided into peripheral models created by direct damage to the bladder[24], and central models created by damage to the spinal cord, brainstem or higher centers[25, 26]. The most common model of PBOO is based on reduction of the urethral diameter by ligation of the urethra[27] and we created PBOO model mice using this method. These mice had ischemia in the bladder at the early stage after creation of the model. Subsequently, this ischemia induced high-pressure urination and bladder dysfunction (DO), and excessive oxidative stress and inflammation at the following stage. These results indicate that PBOO model mice have similar findings to those in human pathophysiology. Our study showed ischemic changes in the bladder at the early stage after creation of the PBOO model, and showed the validity of PBOO model mice. But ischemic changes in PBOO model mice tended to gradually decrease. The cause of decreased ischemic changes at over time is not clear, but we presumed that tissue destruction and fibrosis and / or collateral circulation are related to this condition.

Inflammatory changes play a key role in the pathophysiology of DO following PBOO[19, 20]. Kanno *et al.*[28] found that IL-1β induces bladder remodeling in DO following PBOO. IL-1β is a precursor protein produced by activated macrophages[29], and IL-1β and IL-23 induce expression of IL-17, IL-21, and IL-22 by $\gamma\delta$T cells[29]. MMP can also induce bladder remodeling in DO following PBOO[30, 31]. In the current study, high levels of IL-17, IL-23, MMP-3 and MMP-9 were observed in PBOO model mice compared with sham-operated mice, which suggests that the IL-1-related macrophage activation pathway and MMP might be pathophysiological factors in DO following PBOO. IL-2 (a T cell proliferation factor), IL-3 (a hematopoietic growth factor), and periostin (a component of extracellular matrix) also induce bladder disease, including malignancy[32, 33], and the current study also suggested that IL-2, IL-3 and periostin may be pathophysiological factors in DO following PBOO. Our results also showed the expression of IL-2, IL-3, IL-17A, IL-23, MMP-

3, MMP-9 and periostin in the pathophysiology of DO following PBOO, and T-lymphocytes related cytokines and monocytes and macrophages related cytokine may play a key role in the pathophysiology of DO following PBOO.

However, the various kinds of cytokines and chemokines that mentioned above paragraph may not be a suitable biomarker to monitor the pathophysiology of DO following PBOO in the early stage, and the study of the target marker focused on the pathophysiology of DO following PBOO in the early stage is rarely reported. Additionaly, the relevance between inflammatory changes and oxidative stress and / or functional changes are also still unclear. In our study, we analyzed inflammatory changes in the early stage of DO following PBOO using inflammation-related proteome assay at over time, and analyzed the relationship between inflammatory changes and oxidative stress and / or bladder function. In the oxidative stress analysis, ROS which was expressed in the the early stage, and 8-OhdG which was expressed in the following stage were analyzed in our study. Our study showed the increase of CFS family and ROS in the early stage of DO following PBOO, and functional changes such as increase of maximum detrusor pressure were showed at the same time. In previous report, CSF family which is growth factor that induce the clonal growth of hematopoietic progenitor can also induce bladder defense and disease mechanism[34-36]. The reaction of CSF family shows the first step of innate and adaptive immunity, and regulation of homeostasis in various organs including the bladder[34-36]. Our results suggested that the reaction of CSF family may indicate the first step of innate and adaptive immunity, and regulation of homeostasis in DO following PBOO. These findings shows the possibility that CSF family and ROS are associated with pathophysiology of DO following PBOO in the early stage, and contemporaneous expression of inflammatory changes and oxidative stress in the early stage. Therefore, if we can monitor the change of CFS family and ROS in PBOO, we may be able to predict the pathophysiology of DO following PBOO at the early stage.

CONCLUSION

Oxidative stress and inflammatory changes showed contemporaneous increase in pathophysiology of detrusor overactivity following partial bladder outlet obstruction. Especially, CSF family and ROS changes are showed in the early stage, and might be a predict marker in the pathophysiology of DO following PBOO at the early stage.

CONFLICT OF INTEREST

None

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