〈Regular Article〉

Pathological analysis of age-related bladder dysfunction

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ABSTRACT **Introduction:** Globally, over 50% of adults exhibit lower urinary tract symptoms. While aging is a known risk factor, the pathogenesis of age-related bladder dysfunction remains unclear. Recently, changes in oxidative stress were reported to be associated with the development of age-related bladder dysfunction; therefore, we examined the age-related changes in bladder function in an aging mouse model with a focus on oxidative stress.

Materials and Methods: Experimental animals included male C57BL/6J mice aged 20, 40, 60, and 80 weeks. Using 20-week-old mice as the control group, age-related changes in bladder function were verified. Functional changes in the bladder were investigated using a pressure flow study (PFS), and morphohistological changes were analyzed using upright light microscopy and transmission electron microscopy (TEM). Additionally, changes in oxidative stress in the serum and bladder were analyzed using diacron-reactive oxygen metabolite (d-ROM) and biological antioxidant potential (BAP) tests. The localization of oxidative stress in the bladder was analyzed using nitrotyrosine (NTY) and superoxide dismutase 2 (SOD2) immunofluorescence staining.

Results: The PFS demonstrated significantly decreased maximum cystometric capacity and maximum detrusor pressure, as well as increased overcontraction of the bladder sphincter muscle and inadequate reduction in detrusor pressure after voiding with age. Masson's trichrome staining and TEM showed thinning of the transitional epithelium of the bladder, and fibrosis extending from the interstitium to the muscle layer with age. The pixel area ratio of the epithelial layer significantly decreased, while that of the interstitial and muscular layers significantly increased with age in all layers (from epithelial to muscular layers). Oxidative stress analysis revealed a decrease in antioxidant capacity in the serum and bladder, followed by an increase in oxidative capacity, resulting in the overproduction of oxidative stress. Immunofluorescence staining showed that a biphasic decrease in antioxidant capacity (SOD2)

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preceded an increase in oxidative capacity (NTY) with aging. Analysis of the localization of oxidative stress in the bladder using immunofluorescence staining confirmed highly biphasic staining in the epithelial layer and from the interstitial to the muscular layer, and a biphasic decrease in SOD2 preceded an increase in NTY with aging.

Conclusions: A biphasic state of oxidative stress in the bladder epithelial layer and from the interstitial to muscle layer, as well as bladder dysfunction causing a mixture of overactivity and underactivity of the detrusor muscle, were observed with aging. Oxidative stress is preceded by a decrease in antioxidant capacity, followed by an increase in oxidant capacity. These changes suggest that a decrease in antioxidant capacity may serve as a biomarker reflecting the pathology of early-stage age-related bladder dysfunction.

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Key words：Age-related bladder dysfunction, Pathophysiological analysis, Anti-oxidative stress, Antioxidant capacity, Oxidative capacity

INTRODUCTION

Lower urinary tract symptoms are categorized by the International Continence Society (ICS) as storage, voiding, and postvoiding associated with the lower urinary tract[1]. Globally, the prevalence rate of lower urinary tract symptoms among adults is estimated to be > 50%[2, 3]. Multiple factors are associated with the development of bladder dysfunction leading to lower urinary tract symptoms, of which aging is one of the most important[4]; however, its pathogenesis remains unclear. Aging damages mitochondria which involved in energy production at the cellular level and increases intracellular active oxygen species such as reactive oxygen species (ROS)[1-3]. Increase of active oxygen causes a series of oxidative stress reactions that damage lipids, proteins, carbohydrates, and DNA, as a results cell death is lead[1-3]. Recently, it has been reported that changes in oxidative stress are associated with the development of age-related bladder dysfunction[8-12]. It has also been reported that when oxidative stress is more severe and continues for a long time, it may progress to bladder dysfunction[13-15]. From the above reports, oxidative stress changes may play a key role in the pathology of age-related bladder dysfunction. In this study, we examined the functional, morphohistological, and biochemical changes in bladder function with aging using an aging mouse model. We also examined this pathology with a focus on oxidative stress.

MATERIALS AND METHODS

Research approval and experimental animals

The study was approved by the Animal Research Committee of Kawasaki Medical School, Kurashiki, Japan (approval numbers: 20-069 and 22-066) and was conducted according to the guidelines for the care and use of laboratory animals of Kawasaki Medical School. The experimental animals included C57 black 6 (C57BL/6) male mice (Jackson Laboratory, Bar Harbor, ME, USA) aged 20, 40, 60, and 80 weeks. Using 20-week-old mice as the control group, age-related changes in the bladder were verified.

Bladder functional analysis

Functional changes in the bladder were investigated using a pressure flow study (PFS) under inhalation anesthesia with sevoflurane (Pfizer Japan Inc., Tokyo, Japan). The bladder was exposed via a midline incision, and the dome was punctured and drained using a 26G needle (Terumo, Tokyo, Japan).

An extension tube with three stopcocks (Terumo) was connected to the needle, and normal saline was injected into the bladder at 250 μL/min using a syringe pump (KDS200; Brain Science Idea, Osaka, Japan) (Fig. 1). 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). The maximum cystometric capacity (calculated as the first urination time multiplied by 250 μL/min), maximum detrusor pressure (maximum pressure recorded at the first urination), and detrusor overactivity (bladder contraction independent of urination analyzed within one micturition cycle in the control group) were examined.

Morphohistological analysis

Morphohistological changes in the bladder were analyzed via upright light microscopy and transmission electron microscopy (TEM) using excised sections. The bladders were removed using inhalation anesthesia with sevoflurane. Resected bladder tissues used by upright light microscopy 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 ℃. The tissue slides were dewaxed and hydrated using ethanol-graded solutions in water. Upright light microscopy analysis by Masson's trichrome staining was performed using an Olympus BX-53 microscope (Olympus, Tokyo, Japan). Pixel areas of the epithelial, interstitial, and muscular layers were measured using image processing software (ImageJ; National Institutes of Health, Bethesda, MD, USA), and the pixel area ratios of each layer (epithelium, interstitium, and muscle) were calculated. The resected bladder tissues used by TEM were prefixed in 2.5% glutaraldehyde (Sigma Aldrich, St. Louis, MD, USA) overnight at 4 ℃, and washed in 0.1 M cacodylate buffer (Sigma Aldrich) four times every 15 minutes. Washed bladder was postfixed in 1% osmium tetroxide (Sigma Aldrich) for 120 minutes at 4 ℃ and washed in 0.1 M cacodylate buffer (Sigma Aldrich) for 10 minutes. Fixed tissues were dehydrated in an ethanol series, and finally

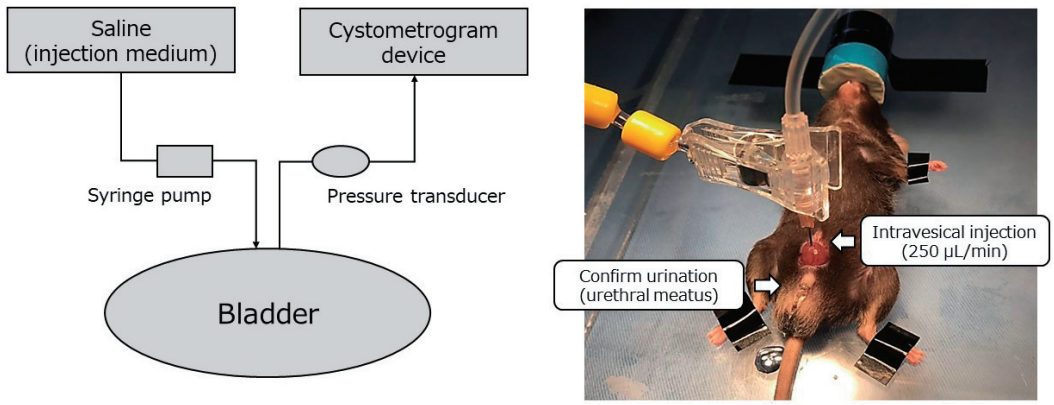

Fig. 1. Pressure flow study mechanism
The bladder was exposed via a midline incision, and the dome of the bladder was punctured and drained using a 26G needle. An extension tube with three stopcocks was connected to the needle, and normal saline was injected into the bladder at 250 μL/min using a syringe pump. Intravesical pressure was measured using a pressure transducer and recorded using a cystometer.

in absolute ethanol. The dehydrated tissues were replaced with propylene oxide (Sigma-Aldrich) twice every 15 min, and with a 1:1 mixture of propylene oxide and epoxy resin (Epon812; Shell Chemical, Monaca, PA, USA) overnight at room temperature. Replaced tissues were embedded in epoxy resin and dried for 72 hours at 60 ℃, and TEM analyses were performed.

Oxidative analysis

Biochemical changes in the serum and bladder were analyzed using the diacron-reactive oxygen metabolite (d-ROM: oxidative power) and biological antioxidant potential (BAP: antioxidant power) tests (Wismerll, Tokyo, Japan). The resected bladder tissue was homogenized with Cell Lysis Buffer (Cell Signaling Technology, Danvers, MA, USA) and phenylmethylsulfonyl fluoride (PMSF; Cell Signaling Technology), and tissue fragments were removed after centrifugation (15,000 × g, 5 min, 5 ℃). Blood was collected from the heart blood, and serum was collected after centrifugation (3,500 × g, 5 min, 5 ℃). The collected bladder and serum samples were analyzed using a redox analyzer (Redoxlibra; Wismerll). The localization of oxidative stress in the bladder was analyzed by immunostaining. Immunohistochemical analysis was performed by immunofluorescence staining (ImmPRESSTM-AP Reagent; Vector Laboratories, Newark, CA, USA). After dewaxing and hydration, blocking was performed for 10 min at room temperature using BLOXALLTM blocking solution (Vector Laboratories). Primary antibodies against indicators of oxidative (nitrotyrosine [NTY]) and antioxidant (superoxide dismutase 2 [SOD2]) power were used after dilution in 2.5% normal horse serum (Vector Laboratories). Anti-NTY antibody (A-21285, diluted 200-fold; Thermo Fisher Scientific, Walthan, MA, USA) and SOD2 (ab68155, diluted 100-fold; Abcam plc, Cambridge, UK) were used. Rabbit IgG control antibody (diluted 2000-fold; Vector Laboratories) was used as a negative control. The primary antibody reaction was performed overnight at 4 ℃. The secondary antibody reaction was performed using the ImmPRESS^{TM}-AP Reagent Anti-Rabbit IgG Polymer Kit (Vector Laboratories) for 30 min at room temperature. The immune substrates used were ImmPACT^{TM} Vector Red Alkaline Phosphatase (Vector Laboratories) for ROS, and Anti-Rabbit IgG (H+L), F(ab')$_2$ fragment (Alexa Floor® 488 Conjugate; Cell Signaling Technology) for SOD. Counterstaining was performed using 4', 6-diamidino-2-phenylindole (DAPI; Vector Laboratories). After encapsulation in VectaMount^{TM} Permanent Mounting Medium (Vector Laboratories), immunohistochemical analysis was performed using a BX-53 microscope (Olympus).

Statistical analysis

Excel Statistics (Microsoft, Redmond, WA, USA) was used for statistical analysis. Differences between 20 weeks and 40-80 weeks were analyzed using Welch's t-test, with $p < 0.05$ considered to indicate a significant difference.

RESULTS

Age-related functional changes in the bladder

The PFS demonstrated a significantly decreased maximum cystometric capacity and maximum detrusor pressure with age (Table 1); it also showed increased overcontraction of the bladder sphincter muscle and inadequate reduction in detrusor pressure after voiding with aging (Fig. 2). Decreased maximum cystometric capacity and overcontraction of the bladder sphincter muscle indicates the development of an overactive bladder with age. Decreased maximum detrusor pressure and inadequate reduction in detrusor pressure after voiding indicates the development of an underactive bladder with aging. These results indicate a mixture of overactive and underactive bladders with aging.

Table 1. Age-related functional changes in the bladder

Maximum cystometric capacity	Mean	SE	p-value
20 weeks old (μL): control	877.75	24.17	–
40 weeks old (μL)	879.50	15.08	0.95340
60 weeks old (μL)	613.75	45.33	0.00365
80 weeks old (μL)	382.25	14.26	0.00001
Maximum detrusor pressure	Mean	SE	p-value
20 weeks old (mmHg): control	31.93	1.90	–
40 weeks old (mmHg)	31.23	1.24	0.76955
60 weeks old (mmHg)	24.90	1.99	0.04306
80 weeks old (mmHg)	13.75	1.60	0.00033

Maximum cystometric capacity and maximum detrusor pressure significantly decreased with aging when compared with 20-week-old mice (control). Data are shown as mean and standard error (SE). Differences in two indicators between 40～80-week-old mice and 20-week-old mice (control) were analyzed by Welch's t-test (n = 4 for each week).

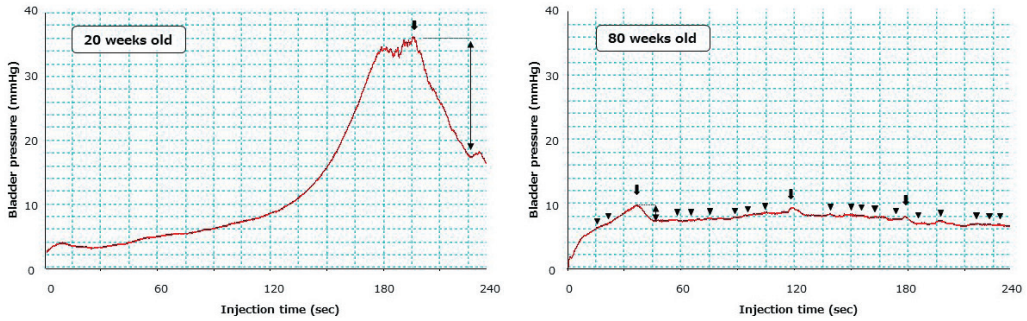

Fig. 2. Age-related functional changes in the bladder
Increased overcontraction of the bladder sphincter muscle and inadequate reduction in detrusor pressure after voiding were observed in 80-week-old mice. Single arrow: urination; double arrow: reduction of detrusor pressure after voiding; triangle: detrusor overactivity

Age-related morphohistological changes in the bladder

Masson's trichrome staining (Fig. 3a, 3b, 3c) and TEM (Fig. 4) demonstrated thinning of the transitional epithelium of the bladder and fibrosis extending from the interstitium to the muscle layer with age. The pixel area ratio of the epithelium in all layers significantly decreased with age (Table 2); by contrast, the pixel area ratios of the interstitial and muscular layers of all layers (from epithelial to muscular layers) significantly increased with age (Table 2).

Age-related oxidative stress changes in the bladder

Oxidative stress assay of the serum and bladder showed decreased antioxidant capacity (BAP test), followed by an increase in oxidative capacity (d-ROM test), resulting in age-related overproduction of oxidative stress (Table 3). Immunofluorescence staining of the bladder confirmed high biphasic staining in the epithelial layer, and from the interstitial layer to the muscular layer (Fig. 5a, 5b); it also demonstrated that a biphasic decrease in antioxidant capacity (SOD2; Fig. 5a) preceded an increase in oxidative capacity (nitrotyrosine; Fig. 5b) with aging.

DISCUSSION

Novelty and summary of this study

In this study, we analyzed the pathophysiology

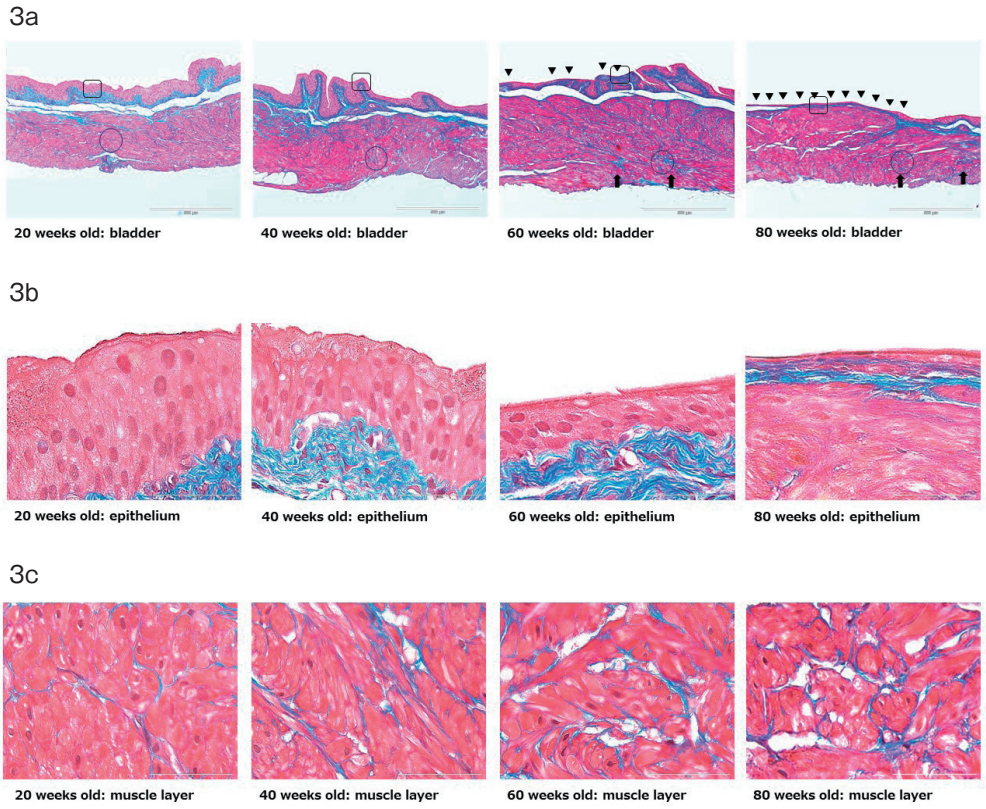

Fig. 3. Age-related histological changes in the bladder
Masson's trichrome staining shows thinning of the transitional epithelium of the bladder (inverted triangle) and fibrosis extending from the interstitium to muscle layer (arrow) with age. Masson trichrome positive (blue) area is a fibrosis area. Fig. 3b highly magnified the square (the transitional epithelium) parts of Fig. 3a. Fig. 3c highly magnified the circular (from the interstitium to muscle layer) parts of Fig. 3a. Fig. 3a; Scale bar: 500 μm; Magnification: 100×. Fig. 3b and 3c; Scale bar: 50 μm; Magnification: 500×

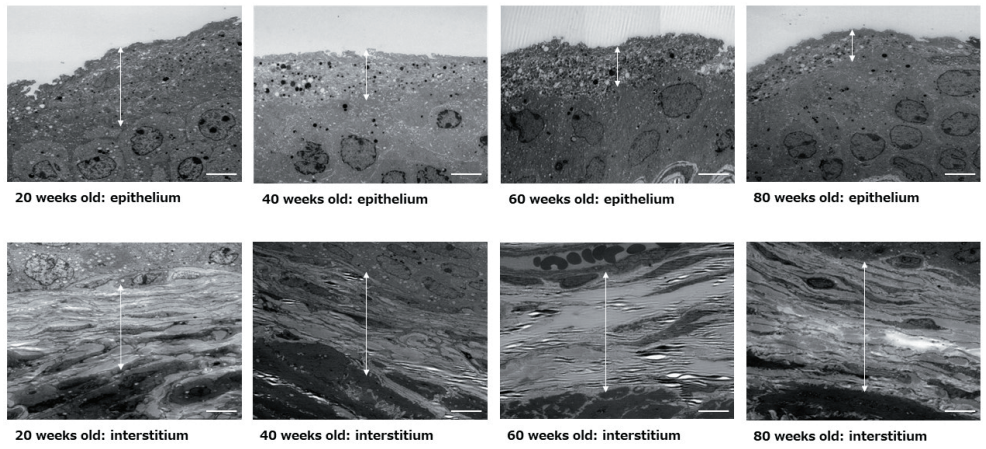

Fig. 4. Age-related morphological changes in the bladder
Transmission electron microscopy revealed thinning of the transitional epithelium of the bladder (epithelium: double arrow) and fibrosis extending from the interstitium to the muscle layer (interstitium: double arrow) with age. Scale bar: 20 μm.

Table 2. Age-related histological changes in the bladder

Epithelial layer / all layer ratio	Mean	SE	p-value
20 weeks old (%): control	6.79	0.26	–
40 weeks old (%)	6.38	0.31	0.35196
60 weeks old (%)	3.81	0.18	0.00023
80 weeks old (%)	3.47	0.18	0.00005
Interstitial layer / all layer ratio	Mean	SE	p-value
20 weeks old (%): control	10.31	0.27	–
40 weeks old (%)	10.33	0.18	0.94543
60 weeks old (%)	11.48	0.15	0.01243
80 weeks old (%)	11.70	0.11	0.00905
Muscular layer / all layer ratio	Mean	SE	p-value
20 weeks old (%): control	82.90	0.19	–
40 weeks old (%)	83.29	0.34	0.36763
60 weeks old (%)	84.71	0.13	0.00050
80 weeks old (%)	84.83	0.20	0.00039

All pixel area ratios of the epithelium in all the layers decreased significantly with age. By contrast, the pixel area ratio of the interstitial and muscular layers in all layers significantly increased with age. Differences in the three indicators between 40～80-week-old mice and 20-week-old mice (control) were analyzed using Welch's t-test (n = 4 for each week)

Table 3. Age-related oxidative stress changes in the bladder

d-ROMs test (serum)	Mean	SE	p-value
20 weeks old (U·CARR): control	36.00	1.73	–
40 weeks old (U·CARR)	38.75	2.84	0.44599
60 weeks old (U·CARR)	61.50	3.75	0.00350
80 weeks old (U·CARR)	75.25	1.75	0.00000
d-ROMs test (bladder)	Mean	SE	p-value
20 weeks old (U·CARR): control	36.25	1.38	–
40 weeks old (U·CARR)	36.75	0.85	0.77006
60 weeks old (U·CARR)	43.50	1.32	0.00900
80 weeks old (U·CARR)	47.00	0.91	0.00128
BAP test (serum)	Mean	SE	p-value
20 weeks old (μmol/L): control	2,388.75	44.60	–
40 weeks old (μmol/L)	2,037.75	91.82	0.02633
60 weeks old (μmol/L)	1,850.75	162.16	0.04938
80 weeks old (μmol/L)	1,351.50	10.44	0.00019
BAP test (bladder)	Mean	SE	p-value
20 weeks old (μmol/L): control	2,579.50	81.06	–
40 weeks old (μmol/L)	1,939.25	68.25	0.00093
60 weeks old (μmol/L)	1,872.50	62.95	0.00046
80 weeks old (μmol/L)	1,648.00	96.82	0.00032

The oxidative stress assay in the serum and bladder showed a decrease in biological antioxidant potential test results, followed by an increase in diacron-reactive oxygen metabolite test results with age. Differences in the two indicators between 40～80-week-old mice and 20-week-old mice (control) were analyzed using Welch's t-test (n = 4 for each week).

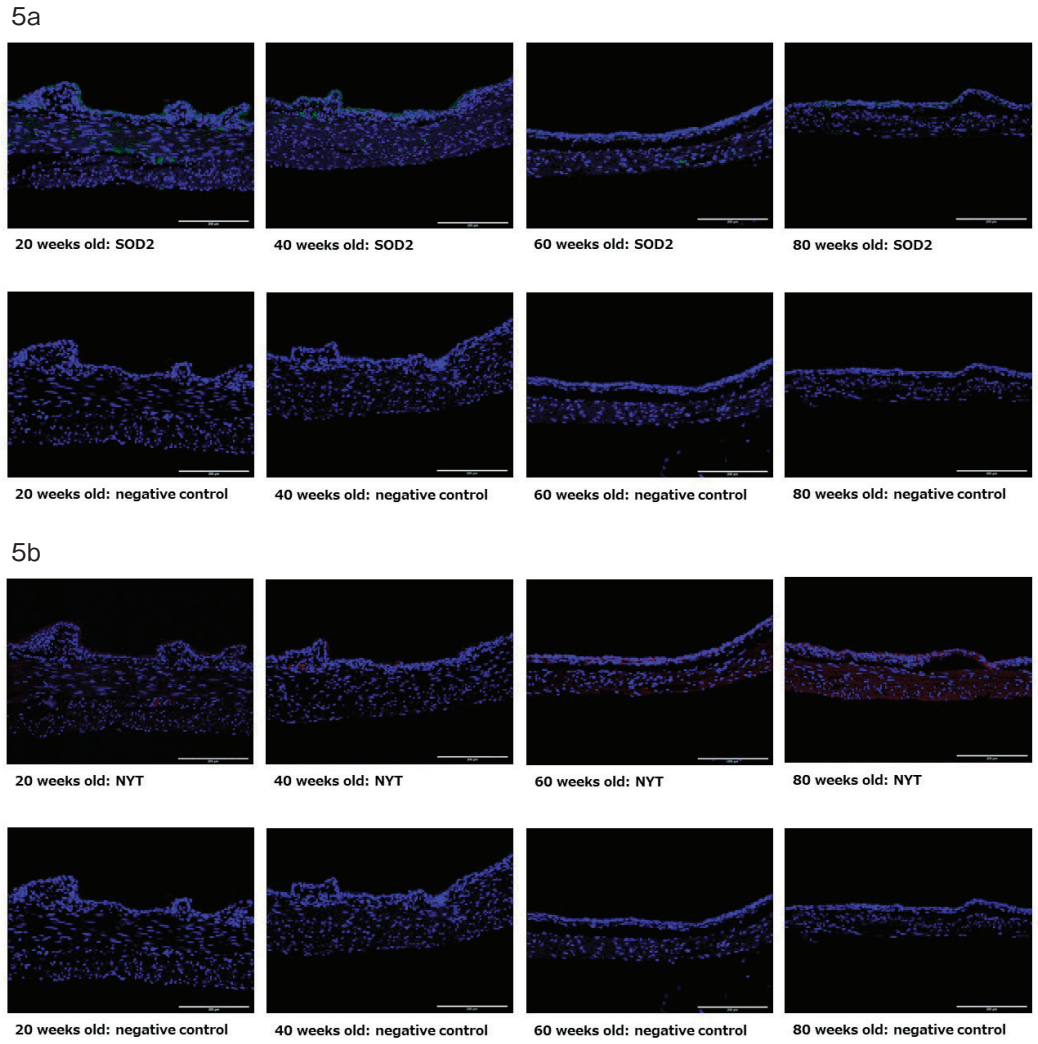

Fig. 5. Age-related oxidative stress changes in the bladder
Immunofluorescent staining of the bladder confirmed high biphasic staining in the epithelial layer and from the interstitial layer to the muscular layer (Fig. 5a, 5b). Immunofluorescence staining showed that a biphasic decrease in antioxidant capacity (SOD2; Fi. 5a) preceded an increase in oxidative capacity (NYT; Fig. 5b) with aging. SOD2, superoxide dismutase 2; NTY, nitrotyrosine. SOD2 positive, green area; NTY positive red area. Scale bar: 200 μm; Magnification: 200×.

of age-related bladder dysfunction in mice using various functional and morphological methods. Furthermore, we subdivided oxidative stress analysis into oxidative and antioxidant capacities. Our results showed a biphasic overproduction of oxidative stress in the epithelial layer, as well as from the interstitial to muscular layer; in addition to bladder dysfunction, this causes a mixture of overactivity and underactivity of the detrusor muscle. The overproduction of oxidative stress is preceded by a decrease in antioxidant capacity, followed by an increase in oxidant capacity.

Pathological changes in age-related bladder dysfunction

An overactive bladder is a symptomatic syndrome

that presents with lower urinary tract dysfunction during the storage phase[16], whereas an underactive bladder presents with lower urinary tract dysfunction during the voiding phase[16]. Although these two symptomatic syndromes are contradictory, aging is a factor in both[17]. Complications of detrusor overactivity (DO) and underactivity (DU) in age-related bladder dysfunction (DO-DU) have been confirmed[17]; however, it has not been clarified whether DO-DU is a simple complication of overactive and underactive bladders, the transition from an overactive to underactive bladder, the transition from underactive bladder to overactive bladder, or a single pathology that develops from the same cause, and the pathogenetic mechanism remains unknown[17, 18]. Our results confirmed the complications of aging in overactive and underactive bladders. Based on the observation that overactive and underactive bladders develop simultaneously, DO-DU may be caused by a single pathology that develops from the same cause. The bladder epithelium permanently contacts urine and repeats damage and repair phases to maintain its functional and organic barrier mechanisms[19–22]. However, when the damage phase exceeds the repair phase due to aging or other causes, the barrier mechanism of the bladder epithelium is disrupted, leading to bladder dysfunction[23]. Bladder dysfunction caused by disruption of the bladder epithelium has been suggested to be associated with overactive bladder[24].

Interstitial fibrosis is also confirmed by morphological changes in bladder dysfunction and has been reported to be associated with a decrease in bladder compliance[25]. Bladder dysfunction caused by interstitial fibrosis has been reported to be associated with an overactive or underactive bladder, and is thought to be strongly associated with an underactive bladder[26]. Our results confirmed the complications of epithelial disruption and interstitial fibrosis associated with aging. DO may be related to epithelial disruption, while DU may be related to interstitial fibrosis.

Oxidative stress changes in age-related bladder dysfunction

The balance between oxidative and antioxidant capacities must be maintained for vital activities[27, 28]; however, if oxidative capacity is increased or antioxidant power is decreased, this balance is disrupted, leading to oxidative stress[27, 28]. In the oxidative stress state, cellular lipids, proteins, cell membranes, and DNA are damaged, and vital activities lead to critical conditions[29–32]. Oxidative stress is involved in the pathology of bladder dysfunction[8–12]. However, these reports[8–12] are based on organic abnormalities and do not simply focus on aging; additionally, they only focused on oxidative capacity, not on the balance between oxidative and antioxidant capacities.

In this study, we analyzed the relationship between the oxidative stress state and bladder dysfunction with aging alone using aging mouse model, confirming that the decrease in antioxidant capacity precedes the increase in oxidative capacity. It may be possible to understand the pathology more accurately at an earlier stage by focusing on changes in antioxidant capacity, rather than oxidative capacity. We also confirmed the localization of the oxidative stress state and biphasic changes in the oxidative stress state between the epithelial layer and interstitial to muscular layer. Additionally, we examined the oxidative stress state in serum and believe that it may be related to changes in the interstitial layer with abundant blood flow in the bladder. Changes in the epithelial layer are presumed to be age-related owing to repeated voiding and storage phases over a long period. These changes were observed in the same layers as the pathological changes, suggesting that oxidative stress is involved in the development of bladder dysfunction.

CONCLUSIONS

A biphasic state of oxidative stress in the bladder epithelial layer and from the interstitial to the muscle layer, as well as bladder dysfunction causing a mixture of overactivity and underactivity of the detrusor muscle, were observed with aging. Oxidative stress is preceded by a decrease in antioxidant capacity, followed by an increase in oxidant capacity. These changes suggest that a decrease in antioxidant capacity may serve as a biomarker reflecting the pathology of early-stage age-related bladder dysfunction.

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CONFLICT OF INTEREST

None

REFERENCES

1）Abrams P, Cardozo L, Fall M, *et al.*: The standardisation of terminology of lower urinary tract function: Report from the standardisation sub-committee of the International Continence Society. Neurourol Urodyn. 2002; 21: 167-178.

2）Coyne KS, Sexton CC, Thompson CL, *et al.*: The prevalence of lower urinary tract symptoms (LUTS) in the USA, the UK and Sweden: Results from the Epidemiology of LUTS (EpiLUTS) study. BJU Int. 2009; 104: 352-360.

3）Irwin DE, Milsom I, Hunskaar S, *et al.*: Population-based survey of urinary incontinence, overactive bladder, and other lower urinary tract symptoms in five countries: Results of the EPIC study. Eur Urol. 2006; 50: 1306-1314.

4）Nishi H. A review of aging and the lower urinary tract: The future of urology. Int Neurourol J. 2021; 25: 273-284.

5）Oakley R, Tharakan B. Vascular hyperpermeability and aging. Aging Dis 2014; 5: 114-125

6）Andersson KE, Nomiya M, Yamaguchi O. Chronic pelvic ischemia: contribution to the pathogenesis of lower urinary tract symptoms (LUTS) : a new target for pharmacological treatment? Low Urin Tract Symptoms 2015; 7: 1-8.

7）Birder LA. Is there a role for oxidative stress and mitochondrial dysfunction in age-associated bladder disorders? Ci Ji Yi Xue Za Zhi 2020; 32: 223-226.

8）Khosla L, Gong S, Weiss JP, *et al.*: Oxidative stress biomarkers in age-related lower urinary tract disorders: A systematic review. Int Neurourol J. 2022; 26: 3-19.

9）Miyata Y, Matsumoto T, Mitsunari K, *et al.*: A review of oxidative stress and urinary dysfunction caused by bladder outlet obstruction and treatments using antioxidants. Antioxidants (Basel). 2019; 8: 132.

10）Greenland JE, Hvistendahl JJ, Andersen H, *et al.*: The effect of bladder outlet obstruction on tissue oxygen tension and blood flow in the pig bladder. BJU Int. 2000; 85: 1109-1114.

11）Levin RM, O'Connor LJ, Leggett RE, *et al.*: Focal hypoxia of the obstructed rabbit bladder wall correlates with intermediate decompensation. Neurourol Urodyn. 2003; 22: 156-163.

12）Kalorin CM, Mannikarottu A, Neumann P, *et al.*: Protein oxidation as a novel biomarker of bladder decompensation. BJU Int. 2008; 102: 495-499.

13）Nomiya M, Yamaguchi O, Akaihata H *et al.*: Progressive vascular damage may lead to bladder underactivity in rats. J Urol 2014; 191: 1462-1469.

14）Yoshida M, Yamaguchi O. Detrusor underactivity: the current concept of the pathophysiology. Low Urin Tract Symptoms 2014; 6: 131-137.

15）Shimizu S, Nagao Y, Kurabayashi A *et al.*: Aging-related severe hypertension induces detrusor underactivity in rats. Life Sci 2021; 283: 119855.

16）Chapple CR, Osman NI, Birder L, *et al.*: Terminology

report from the International Continence Society (ICS) Working Group on Underactive Bladder (UAB). Neurourol Urodyn 2018; 37: 2928-2931.

17) Mancini V, Tarcan T, Serati M, *et al.*: Is coexistent overactive-underactive bladder (with or without detrusor overactivity and underactivity) a real clinical syndrome? ICI-RS 2019. Neurourol Urodyn 2020; 39 (Suppl 3): S50-S59.

18) Chancellor MB, Bartolone SN, Lamb LE, *et al.*: Underactive bladder; Review of progress and impact from the International CURE-UAB Initiative. Int Neurourol J 2020; 24: 3-11.

19) Garcia MA, Nelson WJ, Chavez N. Cell-cell junctions organize structural and signaling networks. Cold Spring Harb Perspect Biol. 2018; 10: a029181.

20) Buckley A, Turner JR. Cell biology of tight junction barrier regulation and mucosal disease. Cold Spring Harb Perspect Biol. 2018; 10: a029314.

21) Winder M, Tobin G., Zupančič D, *et al.*: Signalling molecules in the urothelium. Biomed Res Int. 2014; 2014: 297295.

22) Osborn SL, Kurzrock EA. Production of urothelium from pluripotent stem cells for regenerative applications. Curr Urol Rep. 2015; 16: 466.

23) Parsons CL. The role of the urinary epithelium in the pathogenesis of interstitial cystitis/prostatitis/urethritis. Urology. 2007; 69 (4 Suppl): 9-16.

24) Moore CK, Goldman HB. The bladder epithelium and overactive bladder: What we know. Curr Urol Rep. 2006; 7: 447-449.

25) Kim SJ, Kim J, Na YG, *et al.*: Irreversible bladder remodeling induced by fibrosis. Int Neurourol J. 2021; 25 (Suppl 1): S3-S7.

26) Santos-Pereira M, Charrua A. Understanding underactive bladder: A review of the contemporary literature. Porto Biomed J. 2020; 5: e070.

27) Sies H, Berndt G, Jones DP. Oxidative stress. Annu Rev Biochem. 2017; 86: 715-748.

28) Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature. 2000; 408: 239-247.

29) D'Autréaux B, Toledano MB. ROS as signaling molecules: Mechanisms that generate specificity in ROS homeostasis. Nat Rev Mol Cell Biol. 2007; 8: 813-824.

30) Bhat AH, Dar KB, Anees S, *et al.*: Oxidative stress, mitochondrial dysfunction and neurodegenerative diseases; A mechanistic insight. Biomed Pharmacother. 2015; 74: 101-110.

31) Brown DI, Griendling KK. Regulation of signal transduction by reactive oxygen species in the cardiovascular system. Circ Res. 2015; 116: 531-549.

32) Valko M, Leibfritz D, Moncol J, *et al.*: Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007; 39: 44-84.