Distribution of Interstitial Cells of Cajal in Menopausal Rat Urinary Bladder Showing Detrusor Overactivity

Sun-Ouck Kim, Seung-Hee Song, Kyu-Youn Ahn and Dong-Deuk Kwon

Department of Urology and Anatomy, Chonnam National University Medical School, Gwangju, Korea

Purpose: Recent studies have showed that interstitial cells of Cajal (ICCs) are widely distributed in the genitourinary tract and have suggested their involvement in spontaneous electrical activity and muscle contraction. The purposes of this study were to investigate the effect of estrogen on ICCs in rat urinary bladder from the detrusor overactivity induced by ovariectomy.

Materials and Methods: Female Sprague-Dawley rats (230-240 g, N=60) were divided into three groups: control (N=20), bilateral ovariectomy (Ovx, N=20), and bilateral ovariectomy followed by subcutaneous injections of 17 β-estradiol (50 mg/kg/day, Ovx + Est, N=20). After 4 weeks, urodynamic studies measuring contraction interval and contraction pressure were done. The cellular localization of ICCs was determined by immunohistochemistry in the rat urinary bladder.

Results: Filling cystometry studies demonstrated a reduced interval between voiding contractions and an increased voiding pressure in Ovx group. The approximate the contraction interval (min) was (3.9±0.25) significantly decreased in the Ovx group compared to the control group (6.7±0.15), which was increased after estrogen treatment (9.7±0.22) (p<0.05). Conversely, the average contraction pressures (mmHg) were increased in the Ovx group (28.9±2.1) compared to the control group (21.2±1.45), and decreased after estrogen treatment (24.8±2.21) (p<0.05). The population of c-Kit immunoreactive ICCs was decreased in both the urothelial and muscle layers in Ovx bladders, which increased to the control value after estrogen treatment.

Conclusions: These results demonstrated an decreased immunoreactivity of ICCs in the menopausal rat model and suggest that the decreased population of ICCs expression may contribute to the modulation of bladder overactivity induced by menopause.

Key Words: Interstitial cell; Urinary bladder; Menopause

Introduction

Detrusor overactivity is a major cause of urinary tract symptoms and commonly seen in older patients with menopausal status in female. Loss of ovarian function after menopause or surgical ovariectomy may lead to physiologic and clinical alterations with adverse effect on the various kinds of organs including genitourinary systems.

*This study was supported by a grant of the Korea Healthcare technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (A091028), Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0069443), and partly by a research grant from the Research Institute of Medical Sciences, Chonnam National University (2010-CURIMS-DR004) and by a grant CR109011-1, Chonnam National University Hospital Research Institute of Clinical Medicine.
Hypoestrogenism promotes alterations in the vagina, urethra and urinary bladder that cause dysfunctional lower urinary tract symptoms [1]. Postmenopausal women are subject to bladder dysfunctions, such as largely detrusor overactivity and stress incontinence [2]. Indeed, estrogen replacement therapy increased blood flow in postmenopausal women and significantly reduced urinary symptoms in menopausal women [3].

A better understanding of the underlying pathophysiology is essential because many patients with menopause suffer from the symptoms itself and related harmful effect on their daily lives and decreased quality of life. The mechanisms behind the development of detrusor overactivity after menopause have not yet been fully clarified. The possible mechanism involved in the release of neurotransmitters or sensory nerve activation for sensing bladder fullness remains to be evaluated. Meanwhile, there is a growing interest in interstitial cells of Cajal (ICCs) in the bladder wall. In regards to bladder sensing and contraction response, it has been suggested that the bladder generates intrinsic autonomic contractions, and these intrinsic contractions are coordinated by a specialized system of ICCs [4].

ICCs have been widely investigated in the gastrointestinal tract, where they are thought to have important physiological functions such as being pacemakers for the generation of nerve impulses and propagation of excitation, driving peristaltic activity throughout the gut and also have a key role acting as intermediaries in the transmission of signals from nerve to smooth muscle [5,6]. ICCs have been identified throughout the urinary tract including the renal pelvis, ureter, bladder and urethra. On the basis of these findings, this study hypothesized that ICCs might be involved in the physiology of detrusor overactivity induced by ovariectomy. This study was undertaken to investigate a possible correlation between the distribution of c-Kit immunoreactive ICCs and detrusor overactivity in a menopausal rat model.

Materials and Methods

1. Experimental model
Female Sprague-Dawley rats (230-240 g, N=60) were divided into three groups: control (N=20), bilateral ovariectomy (Ovx, N=20), and bilateral ovariectomy followed by subcutaneous injections of 17β-estradiol (50 mg/kg/day, Ovx + Est, Sigma Chemical Co., St. Louis, MO, USA N=20). Animals were premedicated with xylazine (2.2 mg/kg, IM) and anesthetized with a zolazepam/tiletamine cocktail (4.4 mg/kg, IM). The control group underwent a sham operation. The Ovx group underwent a bilateral ovariectomy and treated with an oil vehicle. The Ovx + Est group underwent a bilateral ovariectomy, followed by treatment with subcutaneous estradiol daily (50 mg/kg/day) at 7th day after ovariectomy. All experimental animals were kept on a standard diet up until the day before the experiment. The day prior to the experiment, the animals were withheld food. Four weeks after ovariectomy and after 3 weeks of vehicle of hormonal replacement, animals confirmed with estrous cycle through vaginal smear were premedicated with xylazine (2.2 mg/kg, IM) and anesthetized with a zolazepam/tiletamine cocktail (4.4 mg/kg, IM). The protocol for animal surgery was approved by the Ethics Committee of animal care and use in the Chonnam National University Medical School.

2. Cystometrogram
Rats were anesthetized with 1.2 g/kg urethane injected subcutaneously (N=10 in each group). A suprapubic midline incision was performed to expose the bladder, a transvesical catheter with a fire-flared tip (polyethylene catheter-50) was inserted into the dome of the bladder and secured with a ligature, and the abdomen was closed. The catheter was connected to a pressure transducer and syringe pump via a 3-way stopcock to record intravesical pressure and infuse saline into the bladder. After the bladder was emptied, cystometry was performed with saline infused at 0.04 ml/min. The contraction pressure and contraction interval were recorded.

3. Immunohistochemistry for ICCs
The bladder tissue was dissected from both lateral walls of the bladder. The tissue was placed in 4% paraformaldehyde fixative for 16 hours and then processed for washing and dehydration. The
tissue was routinely embedded in paraffin, and 6 μm sections were prepared. Tissues were stained with H&E. Immunohistochemistry was performed using an immunoperoxidase procedure (Vector ABC Kit; Vector Laboratories, Burlingame, CA, USA). The tissue sections were deparaffinized in xylene, dehydrated in a graded series of ethanol, rinsed twice in phosphate-buffered saline (PBS), and then treated with 3% H₂O₂ in 60% methanol for 30 minutes to quench endogenous peroxidase activity. After washing twice (5 minutes) in PBS, the sections were incubated for 12 to 14 hours anti-goat polyclonal SCF R/c-kit antibody (R&D Systems, MN, USA) in PBS with 0.3% bovine serum albumin. In the negative control, the sections were incubated in PBS containing 5% normal goat serum only. The sections were then rinsed 3 times in PBS and incubated sequentially for 30 minutes, each with the biotinylated secondary antibody and the ABC reagent. The sections were then incubated for 5 minutes with the peroxidase substrate solution contained in the kit. Finally, the tissue sections were examined and photographed under a light microscope.

4. Statistical analysis
The results were expressed as the mean±the standard error of mean. The Mann-Whitney test and analysis of variance (ANOVA) were performed for statistical analysis. Differences were considered significant at p<0.05.

Results
All animals survived after 4 weeks of the operation. There was no difference in body weight between groups. The weights of the bladders were similar between groups.

1. Cystometric change
In cystometry performed 4 weeks after the operation, the contraction interval (min, mean±SD) was (3.9±0.25) significantly decreased in the Ovx group compared to the control group (6.7±0.15), which was increased after estrogen treatment (9.7±0.22) (p<0.05) (Figure 1). Conversely, the average contraction pressures (mmHg) were increased in the Ovx group (28.9±2.1) compared to the control group (21.2±1.45), and decreased after estrogen treatment (24.8±2.21) (p<0.05) (Figure 1).

2. Histologic change
In control rats, the urothelium consisted of 4-5 layers of transitional cells and lamina propria. Numerous microvasculatures were observed throughout the lamina propria and muscular layers.
Con Ovx Ovx+Est

**Figure 2.** Distribution of c-Kit-immunoreactive interstitial cells of Cajal (ICCs) in urothelium and detrusor smooth muscle. Kit-immunoreactive ICCs are detected in urothelial areas and along the muscle bundle surrounding the detrusor muscle layers. In Ovx bladders, ICCs immunoreactivity against c-kit was decreased compared with control. After estrogen treatment (Ovx+Est), ICCs were more densely distributed in urothelial and muscle layers than in the Ovx group. ICCs of urothelial area and smooth muscle are displayed at the upper and lower panels, respectively. Horizontal scale bar at the bottom of each figure indicates the magnification power.

Each microvascular structure was surrounded by scattered smooth muscle bundles and connective tissues. In the Ovx group compared to the control, the histology of bladder tissue showed relative thinning of the epithelium and atropic change (Figure 2). In the Ovx + Est group, the above histologic changes were restored to the control level.

### 3. Distribution of ICCs

In control bladders, c-kit immunoreactive ICCs were distributed throughout the bladder wall; figures 2 shows c-kit immunoreactive ICCs from the control group distributed in urothelial and detrusor smooth muscle layers. In bladders from the Ovx group compared to control, the number of c-kit immunoreactive ICCs in the urothelial and detrusor muscle layers was decreased (Figure 2). In Ovx + Est group, ICCs in both area restored to the control value. The panels below are displayed at higher magnification in each group.

### Discussion

This study has demonstrated the altered distribution of c-Kit-immunoreactive ICCs in the bladder following ovariectomy in female rats. Four weeks after surgery, cystometric results showed that ovariectomy bladders exhibited a decreased voiding interval and increased voiding pressure compared to control bladders, and returned to the normal control value after estrogen treatment. In ovariectomy group, histological findings showed decreased thickness of urothelial/basal layer of urothelium and detrusor smooth muscle, where ICCs were predominantly distributed. In estrogen replacement group, ICCs in bladders were more widely distributed than in ovariectomy group.

Urinary tracts are known as sensitive to female sex steroid hormones through many clinical or animal studies. Estrogen receptors throughout the bladder and urethra were investigated, suggesting that estrogen has a role in regulating lower urinary tract function [1,2]. Ovariectomy and estrogen administration induces pronounced alterations in lower urinary tract structure and function in animal studies [7]. Ovariectomy results in bladder mucosa atrophy, decreased detrusor...
smooth muscle mass, decreased bladder compliance, and decreased detrusor contractility. In the present study, increased contraction pressure was observed in ovariectomy group that would not anticipated result, we consider this discrepancy maybe due to the complex response of bladder function such as increased voiding frequency and urgency and these responses could increase the voiding pressure. In clinical setting, estrogen replacement therapy has been used for the treatment of women with lower urinary tract symptoms to control urge incontinence, frequency, and nocturia [3].

Recently, it is suggested that the pathophysiology of bladder overactivity may result from complex interactions between the various kinds of elements which include the bladder smooth muscle cells, nerves and neurotransmitters. ICCs have been considered to be one of the elements of this suggested mechanism, as a sensing organ of urinary bladder, as playing a role in detrusor overactivity induced by sex steroid hormonal alteration.

ICCs act as primary pacemaker cells that generate depolarizing currents into neighboring smooth muscles and coordinate muscle contractions in the gastrointestinal tract, playing a fundamental role in signal transmission from enteric nerves to smooth muscle cells [8]. ICCs identified in the urinary tract are largely located throughout the bladder wall [9], and can be divided into two groups [10,11]. ICCs around urothelial, viewed as myofibroblasts by some authors, are closely located by intramural nerves [12] and ICCs in detrusor smooth muscle layer that are mainly located along the boundary of smooth muscle bundles and between muscle bundles [9]. We found somewhat discrepancy between our findings that showing the expression on the basal layer of urothelium, and previous report showing the expression on the just beneath the basal epithelium, in the lamina propria. Although, there are remained controversy about the exact localization of ICCs in urinary bladder, the possible reasons may be related to the fact that we could not conduct co-staining with vimentin that are positive markers of mesenchymal origin ICCs, which is one limitations of current study.

As the ICCs in the around urothelial space are located close to neuronal innervations, an important relationship between bladder ICCs and neuronal innervation is suggested [11]. The changes in ICCs may account for pathologically increased signal transmission between cells in the bladder wall. Some studies suggest that the change of distribution of ICCs in patients with idiopathic and neurogenic detrusor overactivity was observed in human [13] and the spontaneous contractions of muscle bundles have been enhanced in a rat model of bladder overactivity [14]. Further, there are papers stating that smooth muscle cells contractility is regulated by sex steroid [15]. Cretoiu et al represented that c-Kit immunoreactive ICCs were also stained with estrogen receptor on the same cell in female fallopian tube. They suggested that ICCs could function as steroid sensors, and might be implicated in fallopian tube motility [16]. In recent years NO has gained increasing recognition as an important neurotransmitter and cell mediator with a broad range of functions in the lower urinary tract. Common expression of NO-mediated cGMP of ICCs in the bladder have been shown, and both urothelium and detrusor smooth muscle have stained for cGMP [12]. The changes from hormonal alteration might result in alterations to the intrinsic activity of cells, the distribution of this activity within the bladder wall and its sensitivity to excitatory and inhibitory regulators. The limitation of this study is that the precise functional activity of ICCs has not been fully unveiled. We could not investigate the changes in electrical activities of ICCs in the urothelial area and detrusor smooth muscle of the bladder. Further understanding of the pathophysiology of ICCs would give a further insight into the cellular mechanisms of detrusor overactivity by hormonal alteration.

Conclusions

The present study showed a decrease in ICCs in the urothelial area and detrusor smooth muscle in menopausal rat urinary bladders which exhibited bladder overactivity. The expression of ICCs might be involved in signal transmission from the urothelium to afferent nerves and de-
Interstitial Cells of Cajal in Menopausal Rat Bladder

Interstitial Cells of Cajal in Menopausal Rat Bladder

trusor smooth muscle, and they could be new potential targets for the pharmacological treatment of bladder dysfunction induced by sex hormonal alteration. Furthermore, the characteristics of a heterogeneous population of ICCs with distinctive lesions of the bladder wall including lamina propria and detrusor muscle requires much research.

References

10) Sui GP, Rothery S, Dupont E, Fry CH, Severs NJ. Gap junctions and connexin expression in human subendothelial interstitial cells. BJU Int 2002;90: 118-29
13) Biers SM, Raynard JM, Doore T, Brading AF. The functional effects of a c-kit tyrosine inhibitor on guinea-pig and human detrusor. BJU Int 2006;97: 612-6