Persistent Detrusor Overactivity in Rats after Relief of Partial Urethral Obstruction

Long-Hu Jin,1 Karl-Erik Andersson,4 Jeong-Uk Han,2 Yong-Hyun Kwon,1 Chang-Shin Park,3 Hwa-Yoan Shin,1 Sang-Min Yoon,1 Tack Lee1*

Departments of 1Urology, 2Anesthesiology and 3Pharmacology, Inha University College of Medicine by BK 21 project, Incheon, Korea; and 4Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine, Winston Salem, NC, USA

*Corresponding author. : Tack Lee, M.D., Ph.D.
Department of Urology, Inha University College of Medicine by BK 21 project, Jung-Suk Build. 5th Floor, 7-241, 3-Ga, Shinheung-Dong, Jung-Gu, Incheon, 400-711, South Korea
Phone: +82-32-890-3448
Fax: +82-32-890-3097
E-mail address: lt11@inha.ac.kr

Word Count: 4179

Copyright © 2011 by the American Physiological Society.
Abstract

Detrusor overactivity (DO) persists after prostatectomy in 20% to 25% of patients with benign disease. Assuming that nonvoiding contractions (NVCs) can be used as a surrogate for DO in humans, the rat model of obstruction/deobstruction may allow us to study the pathophysiology of persistent DO after deobstruction. We investigated bladder function, with a special focus on NVCs, in rats by use of a new, modified method of obstruction and deobstruction and compared these results with those obtained by use of the conventional method. Seventy female Sprague Dawley rats underwent 1) sham operation (n=10), 2) obstruction by a modified method (Modif-Obs; n=12), 3) obstruction/deobstruction by the conventional method (Conv-Obs/Deobs; n=13), or 4) obstruction/deobstruction by the modified method (Modif-Obs/Deobs; n=35). The Modif-Obs/Deobs animals were divided into subgroups with (DO+) and without (DO-) NVCs. Two weeks after partial urethral obstruction, the animals were deobstructed, and 1 week later cystometry was performed with recording of intra-vesical and intra-abdominal pressures. NVCs were shown in all groups: Modif-Obs (80%), Conv-Obs/Deobs (100%), and Modif-Obs/Deobs (40%). In the Modif-Obs/Deobs group, bladder weight and the muscle/collagen ratio were higher in DO+ than in DO- rats. The Modif-Obs/Deobs group showed no mortality compared with 25% mortality in the
Conv-Obs/Deobs group. The modified method may be more adequate for studying persistent DO after deobstruction, because it resulted in pressure/volume- and DO-related parameters similar to those found in the clinical situation. The persistence of DO after deobstruction may partly be due to irreversible changes in the bladder caused during the period of obstruction.

246 words

Keywords: Urinary bladder, cystometry, detrusor; overactivity,
Introduction

BENIGN PROSTATIC HYPERPLASIA (BPH) occurs as part of the aging process in men and affects more than 50% of the male population over 60 years of age (11). The clinical symptoms of BPH are caused not only by the mass-related increase in urethral resistance but also by pathologic processes in the bladder wall secondary to the bladder outlet obstruction (BOO) (18, 26, 35, 40). In many studies on patients with BOO undergoing surgery for BPH, a significant percentage report persistence of storage (irritative) symptoms, associated with detrusor overactivity (DO) on urodynamic investigation, even if the obstruction was successfully relieved. In fact, DO has been shown to persist after prostatectomy in 20% to 25% of patients treated for benign disease (2, 19, 29, 33).

Rats subjected to BOO will develop hyperactive voiding, characterized by increased frequency and nonvoiding contractions (NVCs), that persists after deobstruction in a proportion of the animals (6, 20, 23, 24, 27, 30). The relations between symptoms, BOO, and bladder function can for obvious reasons not be studied in animal models. However, assuming that NVCs can be used as a surrogate parameter for DO in humans, the rat model of obstruction/deobstruction may offer an opportunity to study the pathophysiology of persistent DO after deobstruction. Many studies have been
performed on the morphological and functional bladder changes that occur after
deobstruction (6, 10, 21, 25, 34), but to the best of our knowledge, no studies have
focused on NVCs and factors that can influence this outcome parameter, which may
have translational value.

To study bladder function in conscious, deobstructed rats by use of conventional
obstructing methods, three abdominal openings are needed: at the time of obstruction, at
the time of deobstruction, and at the time of bladder catheterization (6, 25). The
repeated incisions make it difficult to compare voiding in the obstructed and
deobstructed rats and may increase the complication rate. In fact, deobstructed rats
showed a 25% mortality rate in a study by Chai (6). Therefore, we designed a model of
BOO in female rats in which the knot of an obstructing silk suture is placed in the
vaginal lumen. The ligature is then easily removed through a vaginal approach without
the need to open the abdomen at the time of deligation.

In most animal models, true bladder (detrusor) pressure is not recorded, because this
requires intra-abdominal pressure (IAP) measurement. Lack of IAP recording could be a
source of error when studying DO, because conscious animals are physically active with
consequent increases in IAP/intra-vesical pressure (IVP), and this may occur without
concomitant changes in detrusor activity. In the present study, we therefore measured
IAP and IVP simultaneously by use of a previously described technique (18).

Many studies on the morphological changes in the bladder in the presence of BOO have been performed in experimental animal models and in humans (15, 32, 39). The accumulating evidence obtained from these studies suggests that BOO is followed by an increased thickness of the bladder wall with hypertrophic changes to the detrusor smooth muscle (9, 12, 39) and an altered amount of collagen tissue (8, 37, 38).

Although the role of connective tissue in bladder function has not been clarified in detail, many previous results suggest that an appropriate balance between these two components of the bladder wall may be of great relevance in achieving normal bladder function (14, 26, 35).

The aim of this study was to further study bladder function, with special focus on NVCs, in obstructed/deobstructed rats. To reduce the influence of confounding factors, we used a modified method of obstruction/deobstruction and simultaneous recording of IVP and IAP. These results were compared with those obtained by use of the conventional method. Furthermore, we attempted to reveal possible changes in the amount of connective tissue relative to detrusor smooth muscle by morphometric analysis in rats with obstruction and obstruction/deligation.
METHODS

Experimental Animals. Adult female Sprague Dawley rats obtained from Orient Bio Inc. (GyeongGi, Korea) and weighing 210 to 250 g were housed in a vivarium with free access to water and food with diurnal light cycling. After surgery, the animals were caged individually and maintained in the same manner. All procedures for animal handling and treatment were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and were approved by the Ethics Committee of the Inha University College of Medicine.

Seventy rats were randomly separated into four groups. The first group consisted of control, sham-operated rats (n=10). The second group underwent a modified method of urethral constriction to produce partial bladder obstruction (Modif-Obs), which will be described in the following section (n=12). The third group underwent the modified method of urethral constriction, and after 2 weeks, deobstruction through the vaginal route without an additional abdominal incision (Modif-Obs/Deobs; n=35). The fourth group underwent urethral constriction by the conventional method (4, 24, 28) and then deobstruction through an additional abdominal incision after 2 weeks (Conv-Obs/Deobs; n=13). On the basis of the cystometric criteria for the presence of DO during the filling phase, the Modif-Obs/Deobs group was divided into subgroups
showing DO (DO+) or not showing DO (DO-). DO in this study was defined as the occurrence of NVCs.

**Conventional surgical method.** Anesthesia was induced by ketamine (Ketamine 50®, Yuhan Corp., Korea; 75 mg kg$^{-1}$ intraperitoneally) and xylazine (Rompun®, Bayer Korea Ltd., Korea; 15 mg kg$^{-1}$ intraperitoneally). Through a lower midline incision, the bladder was approached and the proximal urethra exposed. A 3/0 Novafil (monofilament polybutester, Davis & Geck, Wayne, NJ) ligature was placed around the urethra and tied in the presence of an intraluminally placed steel rod with a diameter of 0.9 mm. After the knot was tied, the steel rod was removed, the bladder was repositioned, and the abdominal wall was closed.

**Modified surgical method.** Through a lower abdominal midline incision, the retro-urethral space was exposed and a 4-0 silk stay-suture was placed about 3 mm lateral to the urethra to pull up the vaginal epithelium to make it easy to incise. After a small incision was made, the needle of a 3-0 nylon ligature was inserted through the incision, passed via the vaginal space, and taken out laterally on the other side. The nylon ligature was tied gently around the urethra with the vaginal epithelium in the presence of a 1-mm steel rod placed alongside the urethra. After tying the knot was tied, the steel
rod was removed and the two ends of nylon from the knot were pulled down into the incision, and the knot was rolled down and positioned in the vaginal space, which made it easy to remove through the vaginal approach (Fig. 1).

Cystometric investigations were performed without anesthesia 2 weeks after the first surgery in the sham and Modif-Obs groups and 1 week after the deobstruction in the Modif-Obs/Deobs and Conv-Obs/Deobs groups. The catheters were implanted 3 days before the cystometries.

**Intra-vesical and intra-abdominal pressure catheter implantations.** Through a lower abdominal incision, a polyethylene catheter (PE-50; Becton Dickinson & Co., Franklin Lakes, NJ) with a cuff was implanted into the dome of the bladder and held in place with a purse-string suture. To record IAP, a 0.05-mL balloon (Latex, Dawoo Medical, Incheon, Korea) was made on the tip cuff of a PE-50 polyethylene catheter and placed superior to the bladder, as described previously (20). Both catheters were tunneled subcutaneously and anchored to the skin of the back with a silk ligature. The free end of the catheter was sealed. Each rat was housed individually after surgery, and food and water were given ad libitum. Animals were allowed to recover for at least 24 h.
In vivo cystometric investigations. Awake, unrestrained rats were placed in metabolic cages (Nalgene metabolic cage, Nalge Co., Rochester, NY). The indwelling bladder catheter was connected to a two-way valve that was connected to a pressure transducer (Research Grade Blood Pressure Transducer; Harvard Apparatus, Holliston, MA) as well as an infusion pump (PHD 22/2000 Programmable syringe pump; Harvard Apparatus), and the indwelling IAP catheter was connected to another pressure transducer. Micturition volume (MV) was simultaneously recorded by using a fluid collector connected to a force displacement transducer (Research Isometric Transducer; Harvard Apparatus). Continuous bladder infusion was performed with room temperature, normal saline at a rate of 20 mL h\(^{-1}\) in all groups. Data were digitally stored and analyzed at a later time by using Acq Knowledge 3.8.1 software (BIOPAC Systems Inc., Goleta, CA) at a sampling rate of 100 Hz and an MP150 data acquisition system (BIOPAC Systems Inc.).

The values from three reproducible micturition cycles were used for evaluation. IAP was defined as the recorded balloon pressure corrected by subtracting the lowest balloon pressure in each voiding cycle for zeroing. The detrusor pressure (DP) was defined as the IVP minus the IAP. The NVCs during the filling phase were defined as increments of IVP that exceeded 2 cmH\(_2\)O from baseline without simultaneous changes in IAP and
without fluid expulsion from the bladder (Fig. 2).

The following cystometric parameters were investigated: 1) Cystometric pressure (by DP) and volume parameters, including basal pressure (BP), threshold pressure (TP), maximal pressure (MP), MV, residual volume (RV), bladder capacity (BC; MV + RV), and micturition interval (MI). Compliance was calculated according to the formula (P2–P1)/(V2–V1), where P2 represents the pressure of the stable curve just before the bladder contracts and V2 the infused volume at that time. P1 represents the baseline pressure and V1 the corresponding infused volume. 2) Parameters to investigate DO during the filling phase, including time of filling phase (interval between the initiation of saline infusion and micturition), total number of NVCs during the filling phase, frequency of NVCs (per min), and average pressure difference between the peak and the base of the NVC (NVC pressure) (Fig. 2).

**Histologic Examination.** After cystometry, the animals were sacrificed and the bladders were rapidly excised and weighed. Each bladder was separated at the level of the ureteral orifices into the body and the base, and the bladder body was divided vertically into right and left halves. Six tissue specimens from each group were placed in 10% buffered formalin and fixed overnight at 4 °C for histological examination. After
fixation, they were embedded in paraffin and cut in 4-μm sections. Masson trichrome technique was used to assess changes in the relative areas occupied by muscle (red) and collagen (blue) in the bladder wall in the different groups. All selected fields in the bladder wall were photographed, and images were captured with a Zeiss optic microscope (Carl Zeiss, Inc.) and digital capture system (SPOT FLEX FX1520, SPOT™ Imaging Solutions, a division of Diagnostic Instrument, Inc. MI, USA) at 40x magnification. On each field, the areas occupied by smooth muscle and collagen were circumscribed by computer-assisted line drawing. Quantitative analysis of the muscle and collagen in the area enclosed in the line drawing was done with an image analyzer system (NIH Image J 1.34, http://rsb.info.nih.gov/ij/index.html). The sum of the area occupied by smooth muscle and collagen was assumed to be 100% in each specimen. The areas of muscle and collagen were expressed as a percentage of total area.

**Statistical analysis.** The results are given as mean values ± standard errors of the mean (SEM). Normal distributions were confirmed by the Shapiro–Wilks’ W test. Statistical analyses were undertaken with unpaired Student’s *t*-tests or a one-way analysis of variance (ANOVA) with the Tukey post-hoc test for multiple comparisons. The percentage data for muscle and collagen areas were checked for normality using the
Kolmogorov-Smirnov test (p<0.05). The analysis showed that the difference between the distribution of the analyzed data and the normal distribution was not statistically significant (p>0.05). Statistically significant differences were calculated by one-way ANOVA followed by Tukey’s test as for the comparison of percentage data. All analyses were performed with GraphPad Prism, version 5.03, 2009 (Graph Pad Software, San Diego, CA). $P < 0.05$ was considered statistically significant.
RESULTS

During the course of the experiment, two (17%) rats in the Modif-Obs group died, five (14%) in the Modif-Obs/Deobs group died, and four (31%) in the Conv-Obs/Deobs group died. However, the Modif-Obs/Deobs group showed no mortality after deobstruction among the 30 rats that survived the obstruction. The Conv-Obs/Deobs group showed a mortality rate of 25% after deobstruction among the 12 rats that survived the obstruction (Table 1).

Representative cystometric tracings from rats in the sham, Modif-Obs, Conv-Obs/Deobs, and Modif-Obs/Deobs groups are shown in Fig. 3. Both the filling and the voiding phases of micturition could be reproducibly recorded in the animals. In the Modif-Obs/Deobs group (n=30), we subclassified the animals as DO positive (DO+; n=12) or DO negative (DO-; n=18) after deobstruction as judged by simultaneous changes in IAP and IVP.

Body and bladder weights. There were no significant differences in body weight among any of the groups on the day of cystometry (data not shown). All of the groups with obstruction showed an increase in the ratio of bladder (mg) to body (g) weight compared with that in the sham group (p<0.001). In the Modif-Obs/Deobs group, the
DO+ animals showed a higher ratio than did the DO- subgroup (p<0.01) (Fig. 4).

**Morphology.** Microscopically, smooth muscle bundles were separated from each other by loose collagen fibers (Fig. 5A). In the sham group, the average percentage of smooth muscle and collagen over the sum of both was 33.1±5.0% and 66.9±5.0%, respectively. After obstruction, the percentage of smooth muscle (63.4±2.7%) increased (p<0.05), but that of collagen decreased (36.6±2.7%, p<0.05), compared with the sham group. After deligation, the DO+ group showed a higher percentage of smooth muscle (71.5±1.2%, p<0.001) and a lower percentage of collagen (28.5±1.2%, p<0.001) compared with the values during obstruction. The DO- group, however, showed no significant differences in the percentages of smooth muscle (52.3±3.2%, p>0.05) or collagen (47.7±3.2%, p>0.05) percentages compared with the values of the sham and Modif-Obs groups (Fig. 5B).
**Urodynamic pressure- and volume-related parameters.** Compared with that in sham animals, BP was elevated in the Modif-Obs (p<0.05) and Conv-Obs/Deobs (p<0.05) groups, and also in the DO+ subgroup (p<0.01), but not in the Modif-Obs/Deobs or DO- animals. In addition, BP was higher in the DO+ rats (p<0.001) than in the DO- animals (Fig. 6A).

TP was higher in the Modif-Obs (p<0.01) and Conv-Obs/Deobs (p<0.001) groups, but not in the Modif-Obs/Deobs group, than in the sham group. TP was lower in the Modif-Obs/Deobs (p<0.01) and DO- (p<0.01) rats than in Modif-Obs animals. There was no significant difference between the Modif-Obs group and the DO+ subgroup. However, TP was lower in the DO- subgroup than in the Conv-Obs/Deobs group (p<0.01) (Fig. 6B).

The Modif-Obs group did not show any significant difference in MP compared with the sham group, but MP was higher in the Conv-Obs/Deobs (p<0.01) and Modif-Obs/Deobs groups (p<0.05), and in the DO+ subgroup (p<0.01), than in sham animals. MP was lower in the DO- subgroup than in the Conv-Obs/Deobs (p<0.01) and DO+ animals (p<0.01) (Fig. 6C).

Compliance was increased in all obstructed groups compared with the sham group.

Compared with the Modif-Obs animals, compliance was lower in the Conv-Obs/Deobs
animals (p<0.001) and higher in the Modif-Obs/Deobs (p<0.001) group. Compliance was lower in the Conv-Obs/Deobs group than in Modif-Obs/Deobs (p<0.001) rats.

There was no significant difference in compliance between the DO+ and DO-
subgroups (Fig. 6D).

BC was higher in the Modif-Obs (p<0.001) and Modif-Obs/Deobs (p<0.01) groups than in sham animals. This was due to increased RV (p<0.01) in the Modif-Obs group and was associated with an increased MV (p<0.001) in the Modif-Obs/Deobs group. However, compared with the sham rats, the Conv-Obs/Deobs group showed no increase in BC (p>0.05). There was a significant difference in RV between the DO+ and DO-
subgroups (p<0.05), although BC did not differ significantly between these subgroups (Fig. 7). MI was higher in the Modif-Obs (p<0.001) and Modif-Obs/Deobs (p<0.01) groups, but not in the Conv-Obs/Deobs group, than in the sham group. There was no significant difference in MI between the DO+ and DO- subgroups (data not shown).

Parameters to investigate DO during the filling phase. Whereas the sham group showed no NVCs during the filling phase, the Modif-Obs, Conv-Obs/Deobs, and Modif-Obs/Deobs groups did. NVCs were demonstrated in 8 of 10 rats in the Modif-
Obs group (80%), in all animals in the Conv-Obs/Deobs group (100%), and in 12 of 30
rats in the Modif-Obs/Deobs (DO+ subgroup; 40%) group. There were no NVCs in the
DO- subgroup. The Modif-Obs/Deobs group showed a lower frequency of NVCs than
did the Conv-Obs/Deobs group but did not differ significantly from the Modif-Obs
group. DO pressure was lower in the Modif-Obs/Deobs group than in the Modif-Obs
group but did not differ significantly from that in the Conv-Obs/Deobs group (Fig. 8).
DISCUSSION

Even after surgical relief of BOO, 15% to 30% of men experience persistent DO and storage symptoms (2, 29, 33). The reasons for this have not been established. In animal models designed to investigate directly the postobstruction state of the bladder by use of cystometry, the conventional methods of obstruction and deobstruction have a potential for surgical morbidity and mortality owing to the accumulated operation hazards (6).

This increased morbidity and mortality may have consequences for the urodynamic results. Another issue is the question of what, from a translational point of view, is relevant to measure in the animal model. DO is defined by the International Continence Society as a urodynamic observation characterized by involuntary detrusor contractions during the filling phase which may be spontaneous or provoked (1). In cystometric animal models, it is for obvious reasons not possible to establish whether a detrusor contraction is involuntary or not. However, in animals, the presence of detrusor contractions during the filling phase (NVCs) may be a reasonable surrogate of DO for study of the urodynamic changes that occur after bladder outflow obstruction/deobstruction. In this study, therefore, we focused on this parameter.

The occurrence and frequency of NVCs may be influenced by several factors. One is the surgical trauma of the repeated abdominal or urethral surgery involved in the...
conventional experimental approach. We tried to reduce this factor by introducing a modified experimental procedure, which not only increased the survival rate after deobstruction but also resulted in urodynamic findings differing from those previously reported after deobstruction. We also used simultaneous registrations of IVP and IAP in order to record true detrusor activity. The animals were kept obstructed for 2 weeks, because it is known that bladder function stabilizes by 2 weeks after the initiation of partial outlet obstruction (22). In our study, the deobstructed animals were studied 1 week after deobstruction. This may be a short time period for recovery; nevertheless, 60% of the animals studied by our modified approach had "normalized" bladder function without NVCs.

Rats obstructed by means of our approach showed a significant increase in bladder weight, pressure parameters (including BP and TP), volume parameters (including BC and RV), and compliance after obstruction. These findings are consistent with published results from animal models in which obstruction was performed by means of conventional methods (20, 23, 24, 27, 30). In those studies, obstruction was most often produced by placing one or two loose ligatures around the proximal urethra with a rod of a specific diameter that was then removed to avoid consequent ischemic muscular damage. However, the urethra is apt to be injured during the dissection between the
urethra and the vagina and may rupture because the posterior part of the friable urethra is tightly attached to the vaginal epithelium. To solve this problem, we tied a 3-0 nylon loosely around the urethra including the adjacent vaginal epithelium, which may have had a cushioning effect on the compression. We speculate that the obstructing mechanism of this approach is to kink the urethra rather than compress it. By use of this approach, we could avoid one abdominal incision.

We found that in the modified obstruction/deobstruction (Modif-Obs/Deobs) group, 40% of the animals showed NVCs during the filling phase, as verified by the simultaneous measurements of IAP and IVP. This incidence is similar to what is found clinically. In contrast, all rats in the conventional obstruction/deobstruction (Conv-Obs/Deobs) group exhibited NVCs. The Conv-Obs/Deobs group showed high bladder pressures (BP and TP) and high volume parameters (BC and MV) compared with the sham group. The C-Obs/Deobs group also showed lower compliance than did the Modif-Obs and Modif-Obs/Deobs groups.

There was no significant difference in mortality after the obstruction procedure in the Modif-Obs, Modif-Obs/Deobs, and Conv-Obs/Deobs groups. However, after de-ligation, the Conv-Obs/Deobs group showed a 25% mortality rate, compared with no mortality no mortality in the Modif-Obs/Deobs group. Mortality in the Conv-Obs/Deobs group
was similar to that previously reported by Chai et al. (6). Both the differences in mortality and the differences in function between the Conv-Obs/Deobs and Modif-Obs/Deobs groups might be consequences of the less extensive surgery in the Modif-Obs/Deobs animals.

Previous studies (37, 38) showed that obstruction increases the total amount of bladder collagen, but that, due to the increase in bladder mass, the concentration decreases significantly. We confirmed a decrease in the percentage of collagen after obstruction/deobstruction in all groups, except the deobstructed DO- group. These changes are probably caused by increased functional demands by BOO on the bladder, such as an increase in tension or strain. DO+ deligated rats may have functional BOO, possibly as the result of persistent changes in the central regulation (6). The deobstructed DO- group showed values closer to those of the sham group than the obstructed group. This structural "normalization" may be related to the absence of NVCs.

Chai et al. showed persistent hyperactive voiding after relief of obstruction in 20% of rats. They defined hyperactive voiding on the basis of voiding frequency, using a 4-hour period during the light cycle in a metabolic cage without cystometry (6). In their urodynamic investigation, which was not presented in detail, they found that
hyperactive voiders had significantly higher micturition pressures than did normalized voiders and that the volume of urine of urine per void was lower. This agrees with the findings in our DO+ group.

Possible causes for storage symptoms after deobstructive surgery have been suggested, including persistent obstruction (3, 5, 6, 13, 36). In our study, persistent DO could not be attributed to persistent obstruction, because the percentage of RV to BC was significantly decreased from 36% in the Modif-Obs group to 7% in the Modif-Obs/Deobs group. We found that the bladder weight was not decreased after deobstruction, which suggests that the bladder changes during obstruction were not completely reversible. Furthermore, in the Modif-Obs/Deobs group, the DO+ animals showed a higher ratio than did the DO- subgroup, which may be related to the presence of increased detrusor contractions during the filling phase. Even after relief of obstruction, the changed bladder may not return to its original state before obstruction, and this might result in remaining symptoms (35). For example, Kojima et al. (17) showed that their patients with a high ultrasound-estimated bladder weight above the fixed level of 80 g were characterized by irreversible pathological changes in the detrusor.

Measurement of IAP is standard in human cystometry (31) and provides information
on the true detrusor pressure. However, use of this measurement is still limited in
animal studies. Its advantage is that nonvoiding detrusor contractions can be
discriminated from abdominal straining during the filling phase (16, 20). With the
presence of simultaneous changes on the IVP curve and the IAP curve, we classified the
rats as those with or without NVCs. The NVCs were found in 80% of the Modif-Obs
group, in 40% of the Modif-Obs/Deobs group, and in 100% of the Conv-Obs/Deobs
group. The characteristics of the NVCs were significantly different among the three
groups with regard to frequency and pressure. The Modif-Obs/Deobs group showed a
lower frequency of DO than did the Conv-Obs/Deobs group and a lower pressure
amplitude of NVCs than was found in the Modif-Obs group.

In conclusion, in rats deobstructed by means of conventional methods, we observed
changes in bladder function and NVC characteristics that may be related to the surgical
morbidity and mortality resulting from multiple operations. In contrast, rats operated on
by means of our modified approach showed urodynamic changes similar to those found
in clinical conditions. We confirmed that a significant proportion of deobstructed rats
showed a "normalization" of both structural and functional parameters. In animals with
persistent NVCs after deligation, the obstruction may have caused irreversible or slowly
reversible changes in bladder structures (e.g., gap junctions) (7) causing these functional
abnormalities.

Perspectives and Significance

Our comparison of the conventional method with our new method of partial BOO in
female rats, which can subsequently be relieved through the vagina, demonstrates that
surgical morbidity resulting from multiple, accumulating operational hazards can
influence the changes in bladder structure and function caused by outflow obstruction
and deligation. With the use of our new method, some of these hazards can be avoided.
This change in methodology will make it possible to more accurately analyze the
molecular mechanisms responsible for the changes observed after obstruction and
deligation.

Grants

This study was supported in part by grants from the Korea Science and Engineering
Foundation (KOSEF) funded by the Korean government (MOST) (No. R01-2006-000-10850-0) and the Korea Healthcare technology R&D Project, Ministry of Health & Welfare, Republic of Korea (A090715-1002-0000100).
REFERENCES


Correlation between detrusor collagen content and urinary symptoms in patients

27. Mostwin JL, Karim OM, van Koeveringe G, Brooks EL. The guinea pig as a

28. Pandita RK, Andersson KE. Effects of intravesical administration of the K+
channel opener, ZD6169, in conscious rats with and without bladder outflow

29. Peters TJ, Donovan JL, Kay HE, Abrams P, de la Rosette JJ, Porru D,
Thuroff JW. The International Continence Society "Benign Prostatic
Hyperplasia" Study: the botherosomeness of urinary symptoms. *J Urol* 157: 885-


   *Urology* 64: 2-6, 2004.


**FIGURE LEGENDS**

**Fig. 1.** Schematic illustrations of our modified surgical method of partial urethral constriction. A. A 4-0 silk stay-suture is placed about 3 mm lateral to the urethra to pull up the vaginal epithelium to make it easy to incise. After a small incision is made, the needle of a 3-0 nylon is passed through that incision site via the vaginal space and out laterally on the other side. B. The nylon ligature is tied gently but snugly around the urethra with the vaginal epithelium in the presence of a 1-mm steel rod placed alongside the urethra. C. The two ends of the knot are pulled down into the incision through the vaginal space, and the knot is rolled down and positioned in the vaginal space. D. The knot is easily removed through the vaginal approach. The removed nylon is seen in the small box.

**Fig. 2.** Representative cystometric recordings showing NVCs and abdominal straining reflected by changes in intra-vesical pressure (IVP) and intra-abdominal pressure (IAP). A. An NVC was defined as increments of IVP that exceeded 2 cmH$_2$O from baseline without simultaneous changes in IAP and without fluid expulsion from the bladder. The dagger indicates the NVC. Label ‘a’ indicates NVC pressure from the baseline to the
peak of the NVC. B. Note the abdominal straining, defined as elevations in IVP with simultaneous changes in IAP.

Fig. 3. Representative cystometric tracings in conscious rats from various groups. A. Sham-operated rats. B. Rats with partial outlet obstruction by means of the modified method (Modif-Obs). C. Rats obstructed and then deobstructed by means of the conventional method. D. Rats obstructed and then deobstructed by means of our modified method (Modif-Obs/Deobs) showing NVCs during filling. E. Modif-Obs/Deobs rats showing no DO. DP: detrusor pressure.

Fig. 4. Effect of partial outlet obstruction, deobstruction, and NVCs on the ratio of bladder (mg) to body weight (g). Vertical bars represent mean results plus or minus standard error of the mean. Double asterisks indicate $p<0.01$. Triple asterisks indicate $p<0.001$. Modif-Obs: rats with partial outlet obstruction by means of the modified method; Conv-Obs/Deobs: rats obstructed and then deobstructed by means of the conventional method; Modif-Obs/Deobs: rats obstructed and then deobstructed by means of the modified method; DO+: Modif-Obs/Deobs rats showing NVCs; DO-: Modif-Obs/Deobs rats showing no NVCs; NS, not significant.
Fig. 5. Representative light micrographs of a thin section of detrusor (A) and the percentage of smooth muscle or collagen over the total sum (B) in sham animals, rats obstructed by means of our modified method (Modif-Obs), deobstructed rats showing nonvoiding contractions (NVCs) (DO+), and deobstructed rats showing no NVCs (DO-). Scale bar represents 100 μm. Single asterisk indicates p<0.05. Triple asterisks indicate p<0.001. Modif-Obs: rats with partial outlet obstruction by means of the modified method; Conv-Obs/Deobs: rats obstructed and then deobstructed by means of the conventional method; Modif-Obs/Deobs: rats obstructed and then deobstructed by means of the modified method; DO+: Modif-Obs/Deobs rats showing NVCs; DO-: Modif-Obs/Deobs rats showing no NVCs; NS, not significant.

Fig. 6. Effect of partial outlet obstruction, deobstruction, and the presence of DO on pressure-related parameters including basal pressure (A), threshold pressure (B), maximal pressure (C), and compliance (D). Bars represent mean results plus or minus standard error of the mean. Single asterisk indicates p<0.05. Double asterisks indicate p<0.01. Triple asterisks indicate p<0.001. Modif-Obs: rats with partial outlet obstruction by means of our modified method; Conv-Obs/Deobs: rats obstructed and then
deobstructed by means of the conventional method; Modif-Obs/Deobs: rats obstructed and then deobstructed by means of the modified method; DO+: Modif-Obs/Deobs rats showing NVCs; DO-: Modif-Obs/Deobs rats showing no NVCs; NS, not significant.

Fig. 7. Effect of partial outlet obstruction, deobstruction, and the presence of DO on volume-related parameters including bladder capacity (A), voiding (left), and residual volume (right) (B). Bars represent mean results plus or minus standard error of the mean. Single asterisk indicates p<0.05. Double asterisks indicate p<0.01. Triple asterisks indicate p<0.001. Modif-Obs: rats with partial outlet obstruction by means of the modified method; Conv-Obs/Deobs: rats obstructed and then deobstructed by means of the conventional method; Modif-Obs/Deobs: rats obstructed and then deobstructed by means of the modified method; DO+: Modif-Obs/Deobs rats showing NVCs; DO-: Modif-Obs/Deobs rats showing no NVCs; NS, not significant.

Fig. 8. Representative cystometric tracings and comparison of different physical characteristics of DO obtained in conscious rats with partial outlet obstruction (A, D) and deobstruction by modified (B, D) and conventional (C, D) methods. Significant differences are illustrated by *. Modif-Obs: rats with partial outlet obstruction by means of the modified method; Conv-Obs/Deobs: rats obstructed and then deobstructed by
means of the conventional method; Modif-Obs/Deobs: rats obstructed and then
deobstructed by means of the modified method; DO+: Modif-Obs/Deobs rats showing
NVCs; DO-: Modif-Obs/Deobs rats showing no NVCs; NS, not significant.
Table 1. Mortality of the investigated rats according to the subgroup and procedures.

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Modif-Obs</th>
<th>Modif-Obs/Deobs</th>
<th>Conv-Obs/Deobs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Number, Investigation started</td>
<td>10</td>
<td>12</td>
<td>35</td>
<td>13</td>
</tr>
<tr>
<td>Mortality, total</td>
<td>0</td>
<td>2 (16.7%)</td>
<td>5 (14.3%)</td>
<td>4 (30.8%)</td>
</tr>
<tr>
<td>Mortality, after obstruction</td>
<td>0</td>
<td>2 (16.7%)</td>
<td>5 (14.3%)</td>
<td>1 (7.7%)</td>
</tr>
<tr>
<td>Mortality, after deobstruction</td>
<td>-</td>
<td>-</td>
<td>0 (0%)</td>
<td>3 (25%)</td>
</tr>
<tr>
<td>Real Number, Investigated</td>
<td>10</td>
<td>10</td>
<td>30</td>
<td>9</td>
</tr>
</tbody>
</table>

Modif-Obs: rats with partial outlet obstruction by the modified method, Modif-Obs/Deobs: rats obstructed and then deobstructed by use of our modified method, Conv-Obs/Deobs: rats obstructed and then deobstructed by use of the conventional method.
Bladder Wt (mg)/Body Wt (g) Ratio

- Sham
- Modif-Obs
- Conv-Obs/Deobs
- Modif-Obs/Deobs
- DO+
- DO-
A  

B  

C  

D

**Graphs and Data Analysis**

- **Panel A** shows a graph of IVP (intravesical pressure) and IAP (intraperitoneal pressure) over time.
- **Panel B** illustrates similar data with a focus on 10-second intervals.
- **Panel C** presents a continuation of the pressure measurements.
- **Panel D** displays a bar chart comparing different conditions (Modif-Obs, Conv-Obs/DeLig, Modif-Obs/DeLig) with statistical significance indicated by asterisks (NS = non-significant, * = significant).

**Legend**

- **min**⁻¹: minutes per minute
- **cm H₂O**: centimeters of water

**Statistical Significance**

- DO Freq: Normal (NS) at 0 min⁻¹, significant (*) at 2 min⁻¹.
- DO Pr: Normal (NS) across all conditions.

**Notes**

- The graphs and chart are used to analyze and compare various conditions and their effects on IVP and IAP.