ORIGINAL ARTICLE

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Urethral monopolar cauterization: alternative infravesical obstruction model in male rats

Serkan Akan[1*], Hasan Hüseyin Tavukçu[2], Ibrahim Sogut[3], Ayşe Gökçen Sade[4], Yunus Emre Kızılkan[2], Caner Ediz[2], Ömer Yilmaz[2], Haluk Kulaksızoğlu[5]

SUMMARY

OBJECTIVE: We aimed to determine which method gives the most consistent results between urethral monopolar cauterization and standard urethral partial ligation methods for the urethral obstruction model.

METHODS: Thirty male rats were randomly divided into control, partial ligation, and monopolar cauterization groups. Six weeks after experimental procedures, the experimental groups were evaluated cystometrically, biochemically, and histologically.

RESULTS: According to the cystometric results, bladder capacity, baseline bladder pressure, and compliance data of the monopolar cauterization group were higher than those of the partial ligation and monopolar cauterization groups ($p<0.05$ and $p<0.01$, respectively). As a biochemical evaluation, malondialdehyde levels in bladder tissues of group control were higher than partial ligation and monopolar cauterization groups ($p<0.05$ and $p<0.01$, respectively). The collagen type I level of the control group was higher than the partial ligation and monopolar cauterization groups ($p<0.01$ and $p<0.05$, respectively). Collagen type III levels of the monopolar cauterization group were higher than those of the control group ($p<0.01$), but the Collagen type I/Collagen type III and transforming growth factor-β levels of the monopolar cauterization group were significantly lower than those of the control group ($p<0.001$). As a histological evaluation (hematoxylin and eosin), fibrosis in the lamina propria was more prominent in the monopolar cauterization group than in the control group ($p<0.05$). In addition, the muscular thickness was higher in the monopolar cauterization group compared with control and partial ligation groups ($p<0.001$ and $p<0.01$, respectively).

CONCLUSION: The needle-tipped monopolar cauterization of the posterior urethra may be the method of choice for creating a chronic infravesical obstruction model of infravesical obstruction in male rats.

KEYWORDS: Bladder. Cauterization. Fibrosis. Ligation. Urethral obstruction.

INTRODUCTION

The use of animal models mimicking infravesical obstruction-related urinary symptoms and physiological alterations in males has a critical role in evaluating the potential therapeutic methods[1]. Several methods in animal models have been reported since 1984 and female rats have been more frequently used because of their simpler anatomy and the straightforwardness of the procedures due to absence of the accessory sex organs. In the studies with male rats, midprostatic urethral obstruction with retropubic approach was more common[2-4]. However, probable interaction with the intra-abdominal organs increased the morbidity in the retropubic approach.

Melman et al.'s study, in which urethral partial ligation (PL) with perineal approach and known midprostatic obstruction methods were compared in animal models, was the first study including cystometric and histological examinations. As a result of this study, it was reported that the PL method was superior causing less morbidity[5]. The PL method is the standard infravesical obstruction model in male rats and used for many years. However, this may not be an effortless and speedy method because it requires master suturing skills.

A new, simpler, and faster obstruction model has recently been reported as an alternative to the standard PL model[6]. In this study, the obstruction model was defined by the urethral monopolar cauterization (MC) method and partial obstruction was proven by imaging (retrograde urethrography) performed at the end of the second week after the application. However, this novel model has not been compared with the standard PL method yet.

[1]University of Health Sciences, Fatih Sultan Mehmet Education and Research Hospital, Department of Urology – Istanbul, Turkey.
[2]University of Health Sciences, Sultan Abdulhamid Han Training and Research Hospital, Department of Urology – Istanbul, Turkey.
[3]Demiroğlu Bilim University, Medical Faculty, Department of Biochemistry – Istanbul, Turkey.
[4]University of Health Sciences, Sultan Abdulhamid Han Training and Research Hospital, Department of Pathology – Istanbul, Turkey.
[5]Health Hub Specialty Center, Al Futtaim Healthcare Group – Dubai, United Arab Emirates.
*Corresponding author: drserkanakan@hotmail.com
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We compared the standard PL procedure with the promising and applicable new model to determine which one of the two methods produced the most consistent outcome and demonstrate the efficacy and adverse effects of both procedures regarding physiological, histological, and molecular attitudes.

METHODS

Animal model for infravesical obstruction

All experimental protocols were performed according to the University of Health Sciences Animal Care and Use Committee Guidelines (protocol number 2019-07/01). A total of 30 male Sprague-Dawley rats (350–400 g) were randomly divided into 3 groups with 10 rats in each group as follows: control (C), standard urethral PL, and urethral MC. We administered 100 mg/kg ketamine hydrochloride and 30 mg/kg chlorpromazine intraperitoneally for anesthesia induction and used a 23-gauge catheter sheath for transurethral catheterization[7]. Then, we exposed the posterior part of the urethra through a penoscrotal midline incision. Standard urethral PL procedure, which was previously described by Melman and colleagues, was performed in the PL group[5]. In this procedure, a midline vertical incision of 1 cm was made from the penoscrotal junction to the midscrotum to gain access to the bulbous urethra. The urethra was then isolated from the cavernous bodies, and a sterile metal bar of 0.91 mm in diameter was placed on the prostatic urethral surface. The 3-0 polypropylene suture was secured, and the bar was removed leaving the prostatic urethra partially obstructed. A 4-0 silk suture was used to reapproximate the muscle layer, and a 4-0 nylon suture was used to close the skin.

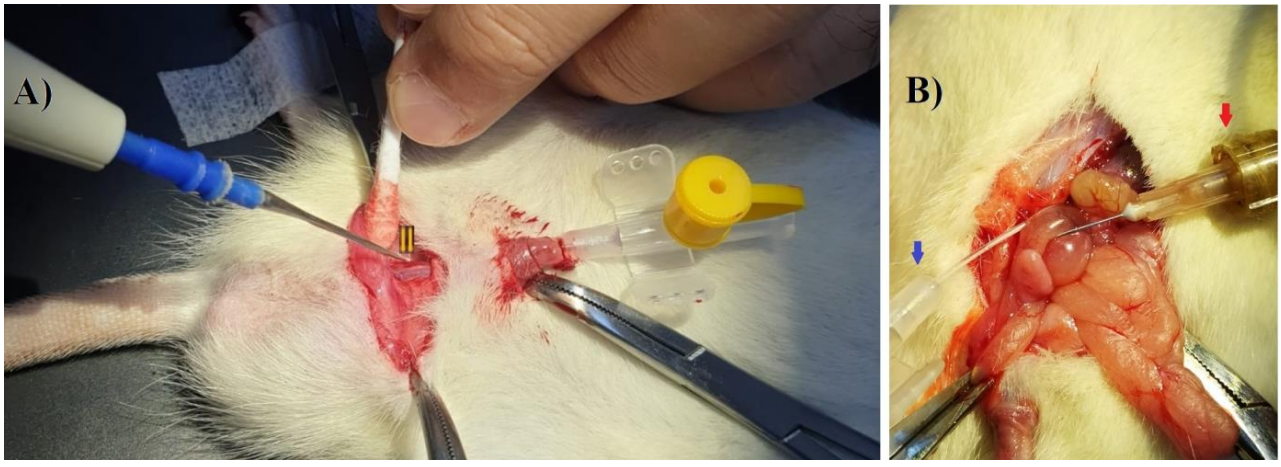
In the MC group, we carried out the monopolar urethral cauterization procedure as described by Tavukcu and colleagues in 2017. In this model,

1. transurethral catheterization with a 23-gauge catheter was performed in the same manner following the anesthetic induction with an intraperitoneal injection of 100 mg/kg ketamine hydrochloride and 30 mg/kg chlorpromazine,
2. the posterior urethra was exposed through a penoscrotal incision of approximately 15 cm,
3. a coagulation current with a level of 10 W was applied for 1 second with the guidance of the catheter at two locations in 2 mm distance, and
4. the procedure was completed after removal of the urethral catheter (Figure 1A).

In both procedures, we closed the skin incision with interrupted monofilament sutures. The C group had only a sham operation.

Cystometric analysis

After 6 weeks, cystometric analysis was performed on all rats. Under general anesthesia by using an intraperitoneal injection of 100 mg/kg ketamine hydrochloride and a 30 mg/kg chlorpromazine, two angiographic catheters were inserted percutaneously into the bladder (24 gauge, 1–2 cm, Baxter Healthcare AS)[8]. One of the catheters was for infusion and the other was for a pressure transducer and an amplifier unit (COMMAT Pharmacology and Physiology Instruments, Ankara, Turkey) (Figure 1B). The amplifier was connected to a data acquisition module (MP35 data acquisition system, Ankara, Turkey).



(A) The procedure of urethral monopolar cauterization. A coagulation current was applied at two locations, each at a 2-mm distance on the posterior urethra (yellow arrows point these locations). (B) The cystometric evaluation by placing the suprapubic catheter and the transducer. Blue arrow: transducer, red arrow: filling catheter.

Figure 1. Catheter applications to experimental animals.

The basal bladder volume was calculated by evacuating the urine from the bladder manually with a syringe. While infusion to the bladder began manually, the other catheter allowed the pressure to be recorded on a computer with the Biopac Student Lab PRO recording software (Biopac Systems Inc., CA, USA). Basal bladder pressure and the maximum capacity bladder pressure were calculated from the records in mmHg and the results were transformed to cm H_2O. The maximum bladder capacity (BC) was noted and the procedure was completed. The bladder compliance was calculated as the bladder pressure per 1 mL

Malondialdehyde levels

We determined the levels of MDA, the end product of lipid peroxidation, in bladder tissue homogenates using the Ohkawa et al.'s method9. The findings were expressed in nanomoles per milligram of protein.

Quantitative Real-Time Polymerase Chain Reaction analysis

Total RNAs were isolated using the RNAzol RT solution (MRC, USA) according to the manufacturer's instructions for quantifying mRNA expression in bladder tissues. After completion of RNA isolation, RNA concentration and purity were calculated using NanoDrop 2000 (Thermo Scientific, USA). For this purpose, 1 µL RNA samples were pipetted in the device for the determination of 260/280 and 260/230 ratios. Concentrations of all RNA samples were equalized before reverse transcription. RNAs were reverse transcribed into cDNA using Script cDNA Synthesis Kit (Jena Bioscience, Germany). The resulting cDNA was amplified by qRT-PCR using qPCR EvaGreenMaster (Solis BioDyne, Estonia). The real-time conditions were carried out on the CFX-96 RT-PCR System (Bio-Rad, USA) as follows: 95°C for 12 min and then 35 cycles of 95°C for 15 s; 55°C for 20 s, and 72°C for 20 s. Relative mRNA transcripts levels were calculated according to the $2^{-\Delta\Delta CT}$ method, and the relative expression of each gene was normalized to that of glyceraldehide-3-phosphate dehydrogenase (GAPDH).

Histological analysis

After the experimental protocol was complete, the animals were sacrificed and the bladder tissues from each of the groups were fixed in 10% formalin for 48 h, paraffin-embedded, and cut into 4 µm sections, and the samples were stained with H&E and Masson's trichrome (MT) for morphological examination. Bladder tissue fragments were investigated for muscular thickness in µm and a semi-quantitative scoring was performed by the pathologist in order to determine the presence of fibrosis and congestion as follows: absent "0," low "1," and high "2".

Statistical analysis

The Graphpad Prism 7 software program (CA, USA) was used for statistical analysis. For normally distributed data, the Tukey's test was used, and for data that was not normally distributed, the Dunn's multiple comparison test was used. The mean±SD and median (interquartile range [IQR]; 25–75th percentile) were used to express the results.

RESULTS

There was not any mortality in the groups in the preoperative, operative, and postoperative periods. The mean duration of the surgical procedures was 14 and 10 min in PL and MC groups. The duration of the procedure was approximately 4 min shorter in the MC group.

The complete cystometric data evaluation was performed to all animals. The maximum BC and the compliance values were significantly higher in the PL and MC groups than the C group. The highest values were in the MC group ($p<0.01$), but the baseline bladder pressure (BBP) was the lowest in the MC group ($p<0.01$) (Table 1).

The PL and MC groups exhibited a significant increase in MDA compared to the C group ($p<0.05$ and $p<0.01$, respectively) (Table 1).

In the bladder tissues, COL1A1 expression was found to be significantly higher in PL and MC groups compared with the C group using qRT-PCR test ($p<0.01$ and $p<0.05$, respectively). When the COL3A1 expression was examined, it was found to be significantly higher only in the MC group compared with the C group in the bladder tissues utilizing the qRT-PCR analysis ($p<0.01$). Transforming growth factor-β (TGF-β) expression was found to be significantly lower only in the MC group compared with the C group ($p<0.001$) (Table 1).

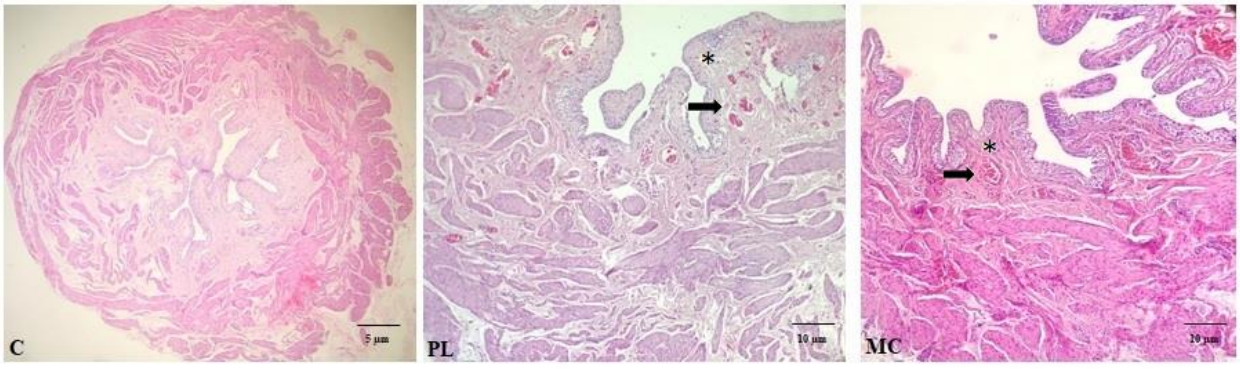
In the prostate tissues, COL1A1/COL3A1 expression was found to be significantly higher only in the MC group compared with the C group, while TGF-β expressions were found to be significantly lower only in the MC group compared with the C group using qRT-PCR test ($p<0.05$ and $p<0.001$, respectively) (Table 1).

In the histological examination of the bladder, the C group demonstrated a regular morphology of the lamina propria and the muscular layer and the PL group had a similar morphology with the C group having slightly increased congestion and fibrosis. Fibrosis in the lamina propria was more prominent in the MC group than in the C group ($p<0.05$). In the MC group, the muscular thickness was higher compared with C and PL groups ($p<0.001$ and $p<0.01$, respectively) (Figure 2).

Table 1. Results of the cystometric and biochemical evaluations of the bladder tissues and results of the biochemical evaluations of the prostate tissues.

Bladder tissues				
Group → cystometric variables ↓	**C**	**PL**	**MC**	**p**
BC	0.25 (0.17–0.3)	0.47 (0.415–0.515)	0.5775 (0.445–0.6825)	C–PL *p<0.05 C–MC **p<0.01
BBP	19.03 (16.65–22.09)	15.63 (13.93–17.33)	15.29 (13.59–18.01)	C–PL *p<0.05 C–MC **p<0.01
MAXBCP	22.09 (20.39–24.13)	20.05 (16.99–22.77)	20.73 (17.67–25.49)	ns
MAXBCP/BBP	3.06 (1.7–4.08)	4.42 (1.7–6.8)	5.44 (3.06–7.82)	ns
Compliance	0.0116 (0.007–0.014)	0.0238 (0.020–0.028)	0.0286 (0.0216–0.0374)	C–PL *p<0.05 C–MC **p<0.01
Biochemical variables ↓				
MDA (nmoL/mg protein)	7.327 (3.368–12.91)	25 (19.55–34.54)	28.31 (22.25–36.32)	C–PL *p<0.05 C–MC **p<0.01
COL1A1/GAPDH	1±0	0.725±0.08385	0.7917±0.1118	C–PL **p<0.01 C–MC *p<0.05
COL3A1/GAPDH	1±0	1.063±0.5301	1.345±0.1674	C–MC **p<0.01
COL1A1/COL3A1	1±0	0.8272±0.3797	0.5917±0.07429	C–MC ***p<0.001
TGF-β/GAPDH	1±0	0.7729±0.2835	0.4829±0.07761	C–MC ***p<0.001
Prostate tissues				
Group → biochemical variables ↓	C	PL	MC	Post-hoc p
COL1A1/GAPDH	1±0	1.733±0.5523	1.403±0.7068	ns
COL3A1/GAPDH	1±0	1.243±0.8879	0.3417±0.1579	ns
COL1A1/COL3A1	1±0	2.788 (0.7591–4.098)	4.235 (3.478–5.169)	C–MC *p<0.05
TGF-β/GAPDH	1±0	1.155±0.8878	0.2767±0.14	C–MC ***p<0.001

C: control; PL: urethral partial ligation; MC: urethral monopolar cauterization; BC: bladder capacity; BBP: baseline bladder pressure; MAXBCP: maximum bladder capacity pressure; MDA: malondialdehyde; COL1A1: collagen type I; COL3A1: collagen type III; TGF-β: transforming growth factor-β; GAPDH: glyceraldehyde-3-phosphate dehydrogenase. Values are given as a mean±SD or median (IQR 25–75th percentile), *p<0.05, **p<0.01, ***p<0.001, ns: no significant.



Group → Variables ↓	C	PL	MC	p
Congestion	**0.0 (0.0-0.25)**	**0.0 (0.0-1.0)**	**0.0 (0.0-1.0)**	**ns**
Fibrosis	**0.0 (0.0-0.0)**	**0.0 (0.0-0.25)**	**0.5 (0.0-1.0)**	**C-MC * p<0.05**
Muscular thickness	**732.2±44.73**	**762.4±37.43**	**837.8±63.33**	**C-MC *** p<0.001 PL-MC ** p<0.01**

C: Control group demonstrated regular morphology of the lamina propria and the muscle tissue, original magnification 5×; PL: Urethral partial ligation. Slightly increased congestion (→) and collagen density. MC: urethral monopolar cauterization, group had the similar morphology with urethral partial ligation group. The bladder wall thickness was greater in the urethral injury group than the others, original magnification 10×.
*In the urethral partial ligation group, original magnification 10×.

Figure 2. Histological sections of the bladders with staining of hematoxylin and eosin

DISCUSSION

This study shows that the needle-tipped MC of the posterior urethra is a simple and reproducible method for creating an effective infravesical obstruction model in male rats. We carried out the MC method in a shorter time and more easily in our study. The PL method, which we have utilized frequently in the previous years, requires experience to dissect posterior urethra and a learning period to suture the rat urethra. Operative mortality and morbidity did not differ between the techniques.

The fibrosis of the bladder involves several molecular mechanisms. Regardless of the etiology, the initial injury is followed by a common morphological pattern including the replacement of the specialized cells by fibroblasts and the collagen deposition which result in alterations in the bladder function[10]. It is well established that the inflammation is usually followed by the tissue fibrosis[11]. It was reported that the production of COL1A1 was increased and COL3A1 was decreased[12]. In our study, COL1A1 expression in the prostate was significantly higher in PL and MC groups compared to the C group. However, the COL1A1-to-COL3A1 ratio, which is a strong indicator of fibrosis, was significantly higher in only MC group according to our QRT-PCR analysis in prostate tissues. Moreover, COL1A1 expression levels in the bladder of the control group were higher than those of the PL and MC groups, while COL3A1 expression levels were lower. COL1A1-to-COL3A1 ratio and TGF-β expression levels in the bladder of the control group were higher than the MC group. In addition, histological analyses of the bladder demonstrated more apparent fibrosis and congestion in the lamina propria and a slightly higher muscular thickness in the MC group than in the other groups. These results in the MC group can be interpreted to be consistent with the detrusor hypertrophy in the compensatory response to the infravesical obstruction[13].

In the development of the fibrosis, TGF-β is shown to be a key factor[14]. Lower TGF-β expression in the first few hours after the infravesical obstruction with a concomitant increase after the onset of chronicity of the injury and tissue fibrosis was reported in studies[13,15,16]. In our study, TGF-β expression in the prostate and bladder tissues was lower in only the MC group compared with the C group. These results are compatible with results of bladder tissues in our previous study as urethral injury rat model[6]. Although in that study COL1A1-to-COL3A1 ratio was significantly higher than C group on the 14th day of injury while in our recent study we investigated tissues in the sixth week of surgery. In our recent study, COL1A1-to-COL3A1 ratio and TGF-β expression were significantly lower than C group which might be interpreted as chronic stage of fibrosis.

The probable role of the oxidative stress on the physiopathology of the bladder dysfunction remains controversial. Previous studies showed that an ischemic period in the bladder, which is subjected to an acute distention, was followed by a reperfusion period resulting in free radical production and these studies speculated about a probable association of the reperfusion period with the chronic infravesical obstruction[17,18]. In these reports, MDA, which is the end product of the lipid peroxidation caused by free radicals, was used as a marker of cell membrane damage and disruption. The levels of MDA in the bladder tissues were significantly higher in the PL and MC groups than in the C group in our study. The highest level of MDA in the MC group demonstrates that this procedure is more thriving than the PL to create a chronic obstruction model. Bisogni et al. investigated total antioxidant status (TAS) in bladder tissue was high in infravesical obstruction model of rats[8]. The levels of the biomarkers of the oxidative stress were higher in the MC group compared with the C group.

When the cystometric measurements were examined, BC was significantly higher in both infravesical obstruction models than in the C group. This result was consistent with the results of Melman and colleagues' study, which compared the PL group with the C group and found that the BC was significantly higher in the former[5]. In Bisogni and colleagues' study, in which the infravesical obstruction model was created by placing a 2-mm silver ring around the bladder neck, the BC did not differ between groups[8]. Another important finding in our study was that the lowest mean BBP was found in the MC group.

Technique of the cystometric analysis is another important issue. Conscious rats are more preferred for cystometric analysis and suprapubic catheters are used[19]. However, for this purpose, bladder tissue is needed to be perforated and sutured thereafter. This might make histological and physiological results vague by impairing bladder function and causing inflammation[19]. If cystometric analysis under general anesthesia is preferred as in our study, transurethral catheterization is performed. Therefore, perforation or saturation of the bladder is not carried out. However, anesthetic agents can also have effects on the bladder function[20].

Among few studies including cystometric analyses in infravesical obstruction models, Melman and colleagues did not assess the bladder compliance in their study. The infravesical obstruction model group was reported to be significantly hypo compliant comparing with the control group in the study of Bisogni and colleagues, in which the cystometric evaluation was performed in the fourth week. In our study, both groups, especially the MC group, were significantly hyper compliant compared with the C group. These results

were consistent with the histological findings of the chronic infravesical obstruction.

According to all results from our cystometric, histological, and molecular analyses, the MC method results in stronger obstruction findings and emerges as an expeditious and simpler alternative to the PL method.

CONCLUSION

Compared with the standard urethral PL method, MC method provides higher rates of fibrosis in the prostate tissue, while bladder tissue results were considered chronic stage of fibrosis. The needle-tipped MC of the posterior urethra may be the method of choice for creating a chronic infravesical obstruction model of infravesical obstruction in male rats.

AUTHORS' CONTRIBUTION

SA: Conceptualization, Data curation, Formal Analysis, Writing – original draft. **HHT:** Conceptualization, Formal Analysis, Writing – original draft. **IS:** Formal Analysis, Writing – original draft. AGS: Formal Analysis. **YEK:** Data curation, Formal Analysis, Formal Analysis. **CE:** Data curation, Formal Analysis. **ÖY:** Formal Analysis, Writing – review & editing. **HK:** Conceptualization, Writing – review & editing.

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