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Comparison of Combination Treatments of Distigmine and either Mirabegron or Solifenacin for Rats with Partial Bladder Outlet Obstruction

Kimio Sugaya[1*], Saori Nishijima[1], Katsumi Kadekawa[1], Katsuhiko Noguchi[1], Katsuhiro Ashitomi[1], Seiji Matsumoto[2], Hideyuki Yamamoto[3]

[1]Southern Knights' Laboratory, Okinawa, Japan
[2]Center for Advanced Research and Education, Asahikawa Medical University, Asahikawa, Japan
[3]Department of Biochemistry, Graduate School of Medicine, University of the Ryukyus, Okinawa, Japan
Email: *sugaya@sklabo.co.jp

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Abstract

Objective: Detrusor hyperactivity with impaired contractility (DHIC) is not an uncommon bladder disorder, and is often difficult to treat. Therefore, using a rat model featuring both urinary frequency and residual urine, we investigated whether an anticholinergic agent (solifenacin) or a β3-agonist (mirabegron) is more suitable to combine with distigmine to treat DHIC. **Methods:** The partial bladder outlet obstruction (BOO) rat model was used. Rats were treated for 2 weeks: BOO/Solifenacin group was treated with 0.1 mg/kg solifenacin (n = 8), BOO/Mirabegron group was treated with 1 mg/kg mirabegron (n = 8), BOO/- group was not drug-treated but was given distilled water (n = 8), and the control group was also given distilled water (n = 8). Then the urethral ligature was removed under urethane anesthesia, and continuous cystometry was performed to evaluate bladder function. Baseline measurements were taken, then distigmine was administered to all groups, and cystometry was performed again to measure changes in bladder function. **Results:** Residual volumes increased in the BOO/- group, and the detrusor contractions were more frequent than that of the control group. Solifenacin treatment did not influence changes, except for threshold pressure, to any cystometric measurements. However, mirabegron treatment decreased the residual volume and residual volume rate; it also decreased detrusor contraction frequency similar to measurements obtained from the control group. Distigmine treatment enhanced detrusor contractions, which resulted in less residual volume, and decreased detrusor contraction frequency in the BOO model. **Conclusions:** The combination of distigmine and mirabegron was

determined to be a better treatment than the combination of distigmine and solifenacin for DHIC.

Keywords

Bladder Outlet Obstruction, Detrusor Hyperactivity with Impaired Contractility, Distigmine, Mirabegron, Solifenacin

1. Introduction

Detrusor hyperactivity with impaired contractility (DHIC) is not an uncommon bladder disorder, which occurs when the detrusor muscle is both overactive during the storage phase and underactive during the voiding phase in the same patient. DHIC is typically diagnosed in patients with bladder outlet obstruction (BOO), enlarged prostates, diabetes, or spinal cord injuries [1] [2] [3], and is often difficult to treat. The treatment for overactive detrusors involves antimuscarinic agents [4] [5]; while α1-blockers and/or cholinesterase inhibitors are used to treat underactive detrusors [6] [7]. However, it is challenging to treat DHIC patients who have both overactive bladder (OAB) and a large residual urine volume; treatment with anticholinergic agents sometimes results in a further increase of residual urine or even urinary retention. If an α1-blocker drug is not sufficiently effective for reducing the residual urine volume [8], then adding a cholinesterase inhibitor, such as distigmine bromide (distigmine), should be considered. Physicians may be hesitant to combine an anticholinergic drug and a cholinesterase inhibitor for an overactive and underactive detrusor. However, in a previously published study, treating rats with spinal cord injuries using a combination of distigmine and the antimuscarinic agent (propiverine hydrochloride) increased detrusor activity in the voiding phase and decreased detrusor activity during the storage phase [9].

In addition to anticholinergic agents, a β3-agonist (mirabegron) is prescribed for overactive detrusor treatment [10] [11]. Mirabegron has a high-binding agonist affinity for β3-adrenoceptors in the bladder, which signals the relaxation of the detrusor muscle to reduce OAB symptoms. β3-adrenoceptors are also present in the urethra [12], and that mirabegron exhibits not only β3-adrenoceptor agonism promoting urethral relaxations, but also selective α1A- and α1D-adrenoceptor antagonism [13]. Therefore, we investigated whether an anticholinergic agent (solifenacin succinate: solifenacin) [4] [5] or a β3-agonist (mirabegron) [10] [11] was more suitable to combine with distigmine for treating DHIC in a rat model that featured both urinary frequency and residual urine [14].

2. Materials and Methods

Adult female Sprague-Dawley rats (n = 32) weighing 210 - 240 g were used. Twenty-four rats underwent BOO-inducing surgical procedures, as previously described [14]. These BOO rats were assigned to 1 of 3 groups: BOO/- group (n

= 8), BOO/Solifenacin group (n = 8), and BOO/Mirabegron group (n = 8). The remaining 8 rats were anesthetized with 2% isoflurane and underwent sham surgery that did not involve tying a 4-0 silk ligature around the urethra (control group). After surgery, all rats were given a subcutaneous injection of 30 mg of ampicillin. The rats were allowed to recover from surgery for one week before treatment began.

The BOO/Solifenacin group was given an intragastric administration of 0.1 mg/kg solifenacin (Vesicare® tablet). Rats in the BOO/Mirabegron group received intragastric administration of 1 mg/kg mirabegron (Betanis® tablet). The dosages were calculated to be similar to those given to patients. For each rat, a tablet was dissolved in 1 mL of distilled water and administered via a lavage once daily for 2 weeks. The control group and the BOO/- group received intragastric administration of 1 mL distilled water once daily for 2 weeks, which was given in the same manner as the groups receiving drug-treated water.

After treatment (3-week postoperatively), the urethral ligature was surgically removed under urethane anesthesia (0.3 g/kg, intraperitoneally and 0.9 g/kg, subcutaneously; total dose of 1.2 g/kg). This removal of the urethral ligature assumed the administration of an α1 blocker to the obstructed bladder in clinical practice. A femoral venous catheter was inserted to administer the distigmine-injected treatments. Rats were given 0.05 mL/min physiological saline infusions into the bladder through an urethral catheter (PE-50, Clay-Adams, Parsippany, NJ, USA) to perform continuous cystometry, as previously described [4].

After bladder contractions and fluid voided from the external urethral meatus had been stable for at least 30 min, the baseline measurements for detrusor activity were measured as listed in **Figure 2** and in previously published work [4]. After the baseline measurements were taken, 0.1 mg/kg distigmine was injected intravenously [4], and we acquired measurements for detrusor activity again and recorded the results in **Figure 2**.

The study protocol was approved by the President of the University of the Ryukyus based on approval from the Institutional Animal Care and Use Committee (No. A2017011 and A2017190).

Results were reported as the mean ± standard error of the mean (SEM). Statistical analysis was performed, as we have previously published, using ANOVA, Tukey's multiple comparison tests, and a paired Student's *t*-test. A $p < 0.05$ indicated statistical significance.

3. Results

Effects of solifenacin, and mirabegron: After 2 weeks of treatment, we acquired baseline measurements for detrusor activity for the 4 groups of rats. At the start of continuous cystometry, many small no-voiding bladder contractions were recorded in the BOO/- group. However, within 30 minutes from the start, these no-voiding bladder contractions almost disappeared. There were several differences observed between the baseline measurements for the BOO/- and

control groups. Shorter interval between detrusor contractions, lower threshold pressure for inducing detrusor contractions, larger residual volumes, and higher residual volume rates were observed in the BOO/- group when compared to the control group (Figure 1 and Figure 2). None of the cystometry baseline measurements were significantly different between the BOO/- and BOO/Solifenacin groups. The threshold pressure level of the BOO/Solifenacin group was the same as that of the control group. When the BOO/- and BOO/Mirabegron groups were compared, there were no significant differences in the baseline cystometric measurements between these groups. The BOO/Mirabegron group had reduced bladder capacity than the BOO/Solifenacin group; in addition, the BOO/Solifenacin group had larger residual volumes. When the control group and the BOO/Mirabegron group were compared, there were no significant differences in cystometric baseline measurements between these groups, except for the threshold pressure. Therefore, mirabegron treatment decreased outflow resistance either at the bladder neck or the urethra in the BOO model.

Effect of distigmine: After the distigmine treatment, we again acquired measurements for detrusor activity for the 4 groups of rats. In the control group, distigmine increased baseline bladder pressure, as well as the maximum pressure and duration of detrusor contractions, but decreased residual volumes and residual volume rates without having any influence on bladder capacity. In the 3 BOO groups, distigmine extended the interval between bladder contractions and the duration of detrusor contractions, and also increased the maximum bladder contraction pressure; while bladder capacity, residual volumes, and residual volume rates decreased. Residual volumes and residual volume rates were lower in

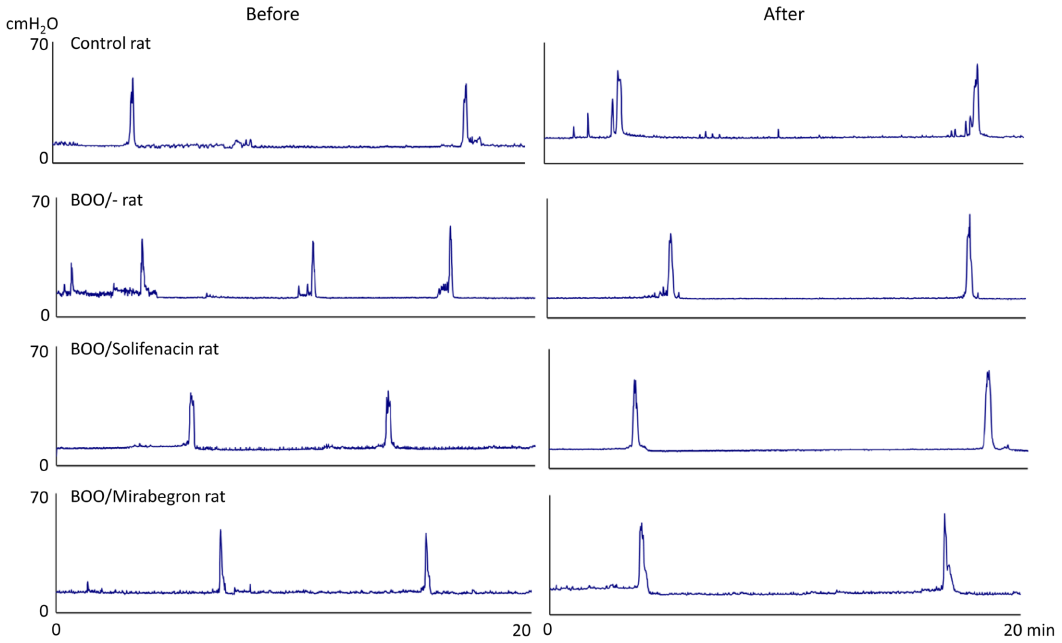

Figure 1. Representative continuous cystometrograms which were acquired before and after distigmine treatment, are shown for the 4 groups in this study: control, BOO/-, BOO/Solifenacin, and BOO/Mirabegron. Before: before distigmine treatment, After: after distigmine treatment.

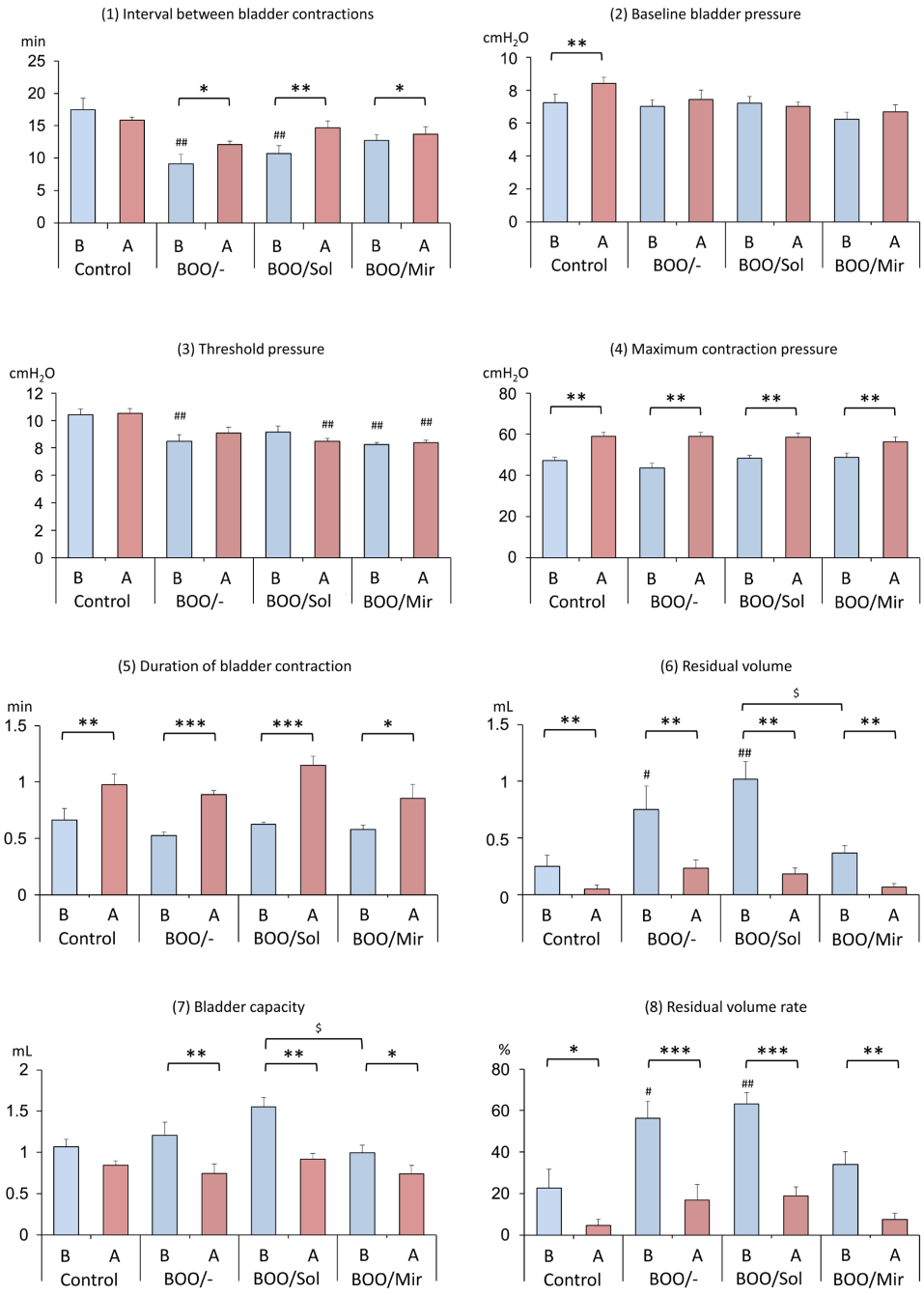

Figure 2. Effects of solifenacin and mirabegron on continuous cystometry measurements in the 4 groups before and after distigmine treatment (n = 8 for each group). BOO/Sol: BOO/Solifenacin, BOO/Mir: BOO/Mirabegron, B: before distigmine treatment, A: after distigmine treatment. Mean ± SEM, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ between before and after intravenous injection of distigmine in each group. #: $p < 0.05$, ##: $p < 0.01$ vs. the control group before or after distigmine treatment. $: $p < 0.05$ between BOO/Solifenacin and BOO/Mirabegron groups before distigmine treatment.

the BOO/Mirabegron group than in both the BOO/- and BOO/Solifenacin groups. However, none of the differences measured after distigmine were statistically significant among the BOO groups.

4. Discussion

The present rat model of BOO showed an increase of residual volume and percent residual volume without any change of bladder capacity or the duration of bladder contractions compared to the control group, resulting in a shorter interval between bladder contractions and a lower threshold pressure inducing bladder contraction. Thus, the BOO model induced mild overactive detrusors. Administration of solifenacin to BOO rats did not influence any cystometric parameters except for threshold pressure. The dosage of solifenacin might be low for rats. However, administration of mirabegron to BOO rats decreased residual volume and residual volume rate, and prolonged the interval between bladder contractions to the level of control group. These results suggest that mirabegron decreased outflow resistance at the bladder neck or urethra in BOO rats. Administration of distigmine prolonged the interval between bladder contractions, and decreased residual volume and residual volume rate without any adverse consequences, especially in the BOO/Mirabegron group. Therefore, distigmine plus mirabegron may be a more suitable combination for overactive bladder associated with residual urine than distigmine plus solifenacin.

As an antimuscarinic agent, solifenacin targets the M3 receptors in the bladder, which mediate detrusor contractions [15], to ameliorate OAB symptoms in patients by significantly decreasing urgency [4] [5]. In addition, others have reported that solifenacin increased bladder capacity in an overactive detrusor rat model [16]. It is thought that urgency results from overactivation of afferent bladder nerves by adenosine triphosphate (ATP) released from the urothelium [17], and solifenacin partly inhibits afferent bladder activity by suppressing non-neuronal release of ATP. Clinically, solifenacin ameliorates OAB symptoms and particularly achieves a significant decrease of urgency [4] [5] [18]. In contrast, β3-adrenoceptor activation in the bladder by mirabegron is known to facilitate bladder filling [19] [20]. β3-adrenoceptor agonists have been reported to increase bladder capacity without altering residual urine volume [21]. The β3-adrenoceptor may be the main receptor facilitating detrusor relaxation [22]. In models consisting of both OAB and BOO, detrusor relaxation is stimulated by β3-adrenoreceptor agonists, like mirabegron, in a dose-dependent manner [19] [23] [24]. Moreover, the β3-adrenoceptor was found to directly inhibit afferent nervous activity in rats after spinal cord transection [21]. Thus, both solifenacin and mirabegron could be expected to relax bladder smooth muscle and directly or indirectly inhibit afferent bladder nervous activity during the storage phase.

To treat patients who have an underactive detrusor with increased residual urine volume, it is necessary to decrease urethral resistance and increase detrusor contraction pressure. The urethral closing pressure could be reduced by treatment with an adrenergic α1-receptor antagonist [8]. In the present study, removal of the urethral ligature prior to cystometry was performed as a treatment similar to reducing urethral resistance with an α1-receptor antagonist. Distigmine treatment enhanced detrusor contractions by increasing the maximum

pressure and length of contractions, which decreased residual urine and extended the interval between contractions. Similarly, in a guinea pig model treated with distigmine, the pressure for detrusor contractions increased to help alleviate underactive detrusor symptoms, but no changes were observed that might exacerbate overactive detrusor symptoms, including urethral closing pressure [25] [26]. These results indicate that distigmine corrects detrusor underactivity during voiding phase without deteriorating storage phase parameters. Thus, it might be logical to use antimuscarinic agents or mirabegron for bladder overactivity causing, while employing distigmine to treat bladder underactivity.

In patients with spinal cord injuries, mirabegron reduced urinary frequency, but some patients displayed an exacerbation of urinary incontinence [27]. Recently, Lee and Kuo reported that mirabegron improved OAB symptoms and decreased post-void residual volume in patients with DHIC [28]. β3-adrenoceptors are also expressed in the urethra [12], and mirabegron exhibits not only β3- adrenoceptor agonism promoting urethral relaxations, but also selective α1A- and α1D-adrenoceptor antagonism [13]. In the present study, administration of mirabegron to BOO rats decreased residual volume and residual volume rate. Thus, our findings in the present study suggest that mirabegron decreases outflow resistance at either the bladder neck or the urethra. Although not significantly different, we determined that the residual volumes and residual volume rates tended to decrease with the combination of mirabegron and distigmine rather than solifenacin and distigmine. Therefore, a combination treatment of distigmine plus mirabegron may be more suitable than a combination treatment of distigmine plus solifenacin for overactive detrusor associated with residual urine. It is challenging to treat DHIC patients. However, the combination treatment of distigmine plus mirabegron during treatment with an adrenergic α1-receptor antagonist might improve detrusor overactivity during the collection phase, reduce residual urine, and improve lower urinary tract symptoms.

5. Conclusion

In conclusion, a combination of distigmine (a cholinesterase inhibitor) and mirabegron (a β3-adrenoreceptor agonist) would be a better treatment for DHIC rather than the treatment combination of distigmine and solifenacin (an antimuscarinic agent).

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

ANOVA = Analysis of Variance
ATP = Adenosine Triphosphate
BOO = Bladder Outlet Obstruction
OAB = Overactive Bladder
SEM = Standard Error of the Mean