Received: 28 June 2019 | Revised: 27 September 2019 | Accepted: 14 October 2019

DOI: 10.1111/luts.12296

ORIGINAL ARTICLE

WILEY

Effects of combined treatment with fesoterodine and mirabegron in a pelvic congestion rat model: Results from in vitro and in vivo functional studies

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Funding information
Pfizer Japan Inc

Abstract

Objectives: To examine the effect of combining a nonselective muscarinic receptor antagonist, 5-hydroxymethyl tolterodine (an active metabolite of fesoterodine), with a β3 adrenoceptor agonist, mirabegron, in a rat model of pelvic congestion.

Methods: The rat pelvic congestion model used female Sprague-Dawley rats with their bilateral common iliac and uterine veins ligated. Expressions of M2 and M3 receptor subtypes in the urothelium and detrusor were detected by real-time polymerase chain reaction assays. The effects of both drugs were investigated on isolated bladder strips contracted by electrical field stimulation. in vivo single cystometry was used to assess the effects of 5-hydroxymethyl tolterodine and mirabegron independently or in combination on bladder capacity, micturition pressure, and threshold pressure.

Results: Pelvic congestion rats showed decreased bladder capacity compared with controls, but micturition pressure and threshold pressure were unchanged. Pelvic congestion model rats also demonstrated an approximately two-fold increase in expression of both M2 and M3 receptor subtypes in the urothelium. Additive relaxant effects of 5-hydroxymethyl tolterodine and mirabegron were observed in vitro in the electrical field stimulation-induced contractions of bladder strips from pelvic congestion rats. In vivo, bladder capacity was increased significantly by a combination of 5-hydroxymethyl tolterodine and mirabegron, with the combined effect exceeding the sum of the effects of monotherapies. Micturition pressure and threshold pressure did not significantly differ between groups.

Conclusions: The combination of 5-hydroxymethyl tolterodine with mirabegron suggests the potential of synergistic effects in a rat pelvic congestion model.

KEYWORDS

drug combinations, fesoterodine, mirabegron, overactive, rats, urinary bladder

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Work was performed at: Pfizer Japan Inc. Shinjuku Bunka Quint Bldg.3-22-7, Yoyogi, Shibuya-ku, Tokyo 151-8589 Japan; Southern Knights' Laboratory Co., Ltd., 1-1-823 Miyagi, Chatan-cho. Nakagami-gun, Okinawa, Okinawa, 904-0113, Japan; Center for Pharma-Food Research, Graduate School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga Shizuoka, 422-8526, Japan.

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1 | INTRODUCTION

Overactive bladder (OAB) syndrome is defined by the International Continence Society as "urinary urgency, with or without urge incontinence, usually with frequency and nocturia" in the absence of urinary tract infection or other obvious pathology.[1] Thus, OAB symptoms tend to feature the following four components: urgency, urinary frequency, nocturia, and urinary incontinence. Because urinary frequency with OAB is associated with a reduction in functional bladder capacity (BC), BC provides a metric by which pharmacologic treatment effects can be measured.

Muscarinic receptor antagonists (antimuscarinics) and β_3 adrenoceptor agonists are the currently recommended pharmacologic treatments for OAB. These two classes of drugs demonstrate distinct mechanisms of action: antimuscarinics reduce detrusor overactivity by binding to muscarinic receptors,[2] whereas β_3 agonists exert a relaxing effect on detrusor muscles by binding to β_3 adrenoceptors.[3] Because these drugs function via different mechanisms, the combination of antimuscarinics and β_3 agonists is expected to increase efficacy compared with monotherapy. Success in this realm has been demonstrated for the combination of solifenacin, a selective muscarinic M3 antagonist, and mirabegron, a β_3 adrenoceptor agonist, which led to significant improvement of storage symptoms when used in combination.[4-7]

Specifically using an M2 inhibitor in combination with a β_3 adrenoceptor agonist may provide additional therapeutic benefit. Acetylcholine (ACh), a neurotransmitter that causes bladder contraction, functions both directly, via muscarinic M3 receptor stimulation, and indirectly, by inhibiting adenylyl cyclase (AC) via stimulation of muscarinic M2 receptors.[8] β_3 adrenoceptor agonists are presumed to cause relaxation of detrusor smooth muscles by activating AC.[9] Therefore, inhibition of the M2 receptor may enhance relaxation caused by activation of the β_3 adrenoceptor in addition to M3 receptor blockade. To test this hypothesis, we examined the effect of combining a nonselective muscarinic receptor antagonist, 5-hydroxymethyl tolterodine (5-HMT), which is an active metabolite of fesoterodine, with mirabegron, a β_3 adrenoceptor agonist. Fesoterodine functions as an orally active prodrug that is converted to the active metabolite 5-HMT by nonspecific esterases. The transformation from fesoterodine to 5-HMT is extremely quick; fesoterodine cannot be detected in plasma after oral administration.[10] 5-HMT is the main active principle of fesoterodine and is a nonselective muscarinic receptor antagonist of subtypes M1 through M5.[11]

Our study used a pelvic congestion (PC) rat model to examine storage dysfunction. The PC rat demonstrates urinary frequency with mild inflammatory changes to the bladder wall.[12] Changes in M2 and M3 receptors were assessed in addition to examining the effects on storage function.

2 | METHODS

2.1 | Rat PC model

Forty-nine female Sprague-Dawley rats weighing 250 to 300 g were used in this study. The study protocol was approved by the president of the University of the Ryukyus based on the judgment of the University's Institutional Animal Care and Use Committee. The PC model involved 49 rats, which were first anesthetized with 2% isoflurane. An incision was made in the lower abdomen, followed by ligation of the bilateral common iliac veins with metal clips and en bloc ligation of the bilateral uterine veins, arteries, and uterine horn at a site adjacent to the ovaries. Dilation of the distal common iliac veins was observed after the ligation. The abdominal incision was closed, and 30 mg of ampicillin was subcutaneously injected. Twenty-four rats were randomly divided into three groups (5-HMT, mirabegron, and combination groups; n = 8 for each). In the sham group, the remaining eight rats were anesthetized with isoflurane, and the bilateral common iliac veins were dissected from the common iliac arteries.

2.2 | M2 and M3 mRNA expression in the bladder mucosa and the detrusor muscle

The expression of M2 and M3 receptor subtypes in the rat PC models was detected as follows. Resected tissues were stored in RNAlater (Qiagen; Hilden, Germany) at −20°C. Bladder mucosa was carefully removed from the bladder wall by removing the tissue adjacent to the smooth muscle layer (n = 6) in RNAlater solution. Total RNA was extracted from each tissue sample using a high pure tissue RNA isolation kit (RNeasy Fibrous Tissue Mini Kit, Qiagen). Complementary DNA (cDNA) was synthesized from sample total RNA using a SuperScript VILO cDNA Synthesis Kit (Invitrogen, Carlsbad, CA, USA) according to manufacturer recommendations. Real-time polymerase chain reaction (PCR) was performed using the TaqMan Universal Master Mix and optimized TaqMan probe sets (Table 1). Samples were amplified, and cycle threshold (Ct) values were measured using the StepOne Real-Time PCR system (ThermoFisher, Waltham, MA, USA). Amplification conditions were 95°C for 20 seconds, followed by 40 cycles of 95°C for 1 second and then 60°C for 20 seconds. The comparative Ct method (2-ΔΔCt) was used to analyze gene expression differences between disease model rats (n = 5) and sham rats (n = 6) with statistical significance determined using the Student's *t* test.

TABLE 1 Quantitative RT-PCR primer sets obtained for expression analyses

Functional class	Genes	ABI assay ID
Housekeeping gene cholinergic receptor	Actb	Rn00667869_m1
	Chrm3	Rn00560986_s1
	Chrm2	Rn02532311_s1

Abbreviations: RT-PCR, real-time polymerase chain reaction.

2.3 | In vitro functional studies: Organ bath studies

The independent and combinatory relaxant effects of 5-HMT and mirabegron on the bladder strips contracted by electrical field stimulation (EFS; 1 Hz) were evaluated as follows. Bladders from PC rats were isolated and placed immediately in ice-cold Krebs solution (NaCl 119 mM, KCl 4.6 mM, $CaCl_2$ 1.5 mM, $MgCl_2$ 1.2 mM, $NaHCO_3$ 15 mM, NaH_2PO_4 1.2 mM, glucose 5.5 mM). Semicircular strips of bladders sized 1 × 2 × 5 mm were prepared from the middle third of the detrusor. These strips were mounted in 5 mL organ baths containing Krebs solution at pH 7.4 maintained at 37°C and constantly gassed with 95% O_2 and 5% CO_2.

Using silk ligatures, these strips were suspended between two L-shaped hooks: one connected to a unit capable of adjusting to passive tension, and the other to a T7-15-240 force transducer (A&D Co. Ltd., Tokyo, Japan). Isometric tension was recorded using a Linearcorder WR3320 (Graphtec Co., Kanagawa, Japan). The strips were subjected to a passive tension of 4 mN and allowed to equilibrate for 45 to 60 minutes before further experiments were conducted.

The treatment concentrations of 5-HMT (1 nM) and mirabegron (100 nM) were set by approximately 20% inhibition of bladder strip contraction by carbachol stimulation, as previously determined in a separate experiment (data not shown). Briefly, 5-HMT dosing was based on data from the literature and the projected rat intravenous dose inferred from human exposure, and effects of mirabegron on normal bladder relaxation were studied in bladder strips from 4- to 8-week-old normal Sprague-Dawley female rats. A relaxing effect of mirabegron was observed from a starting concentration of 10^{-10} M, and the percentage of relaxation increased in a concentration-dependent manner. The curve generated from this observation was used to calculate a dose that induced 20% relaxation. Two platinum electrodes were placed on each side of the strips, and EFS was delivered by an Electronic Stimulator SEN-3301 (Nihon Kohden Co., Tokyo, Japan) in the form of single square-wave pulses at selected frequencies. The frequency, pulse duration, and voltage of stimuli were 1 Hz, 0.1 ms, and 10 V, respectively. In our preliminary experiment, the contractile response to EFS at this stimulation condition was tetrodotoxin (TTX)-sensitive, suggesting a predominantly nerve-mediated response and not a muscle direct response (Yamada et al., unpublished data).

2.4 | Single cystometry

Four weeks after the surgery, eight sham and 24 PC rats were subcutaneously anesthetized with urethane (0.6 mg/kg), after which a polyethylene catheter (PE-50, Clay Adams, Parsippany, NJ, USA) was inserted transurethrally into the bladder. The catheter was connected to a pressure transducer and an infusion pump through a three-way stopcock. A fine polyethylene catheter was inserted into the femoral vein for drug injection. After the isoflurane anesthesia was stopped, rats were placed in restraining cages (Ball man cage, Yamashita Giken, Tokushima, Japan).

For single cystometry, the bladder was filled with physiological saline through the transurethral catheter at a rate of 0.05 mL/min. When the bladder volume induced voiding and the maximum bladder contraction pressure were stable after three to five cycles of single cystometry, vehicle (5% DMA, 5% cremophor, and 90% distilled water, at 1 mL/kg) was administered intravenously to the sham and PC rats. After vehicle injection was confirmed to have no influence on cystometric parameters, mirabegron (0.1 mg/kg, n = 8), 5-HMT (0.01 mg/kg,[13] n = 8), or their combination (n = 8) were administered intravenously to PC rats, and single cystometry was repeated 1 minute after administrations. BC was determined by multiplying the infusion rate (0.05 mL/min) by the elapsed time from injection of physiological saline into the bladder until commencement of urination. Residual volume was directly measured through the transurethral catheter. Voided volume was the value obtained by subtracting the amount of residual urine from the BC.

The following cystometric parameters were investigated: micturition pressure (MP; maximum bladder contraction pressure during micturition), threshold pressure (TP; pressure at which the micturition contraction was initiated), BC (volume of the infused saline prior to that micturition), micturition volume (MV; volume of the expelled urine), and residual volume after micturition. BC was the primary endpoint for the in vivo experiments.

Results are presented as mean values ± SE, and comparisons were made between before and after intravenous drug administration and among groups. Differences within groups were assessed by Dunnett test, Dun's test, and analysis of variance (ANOVA). Differences were considered statistically significant when $P < 0.05$.

3 | RESULTS

3.1 | Expression of M2 and M3 muscarinic receptors mRNA in PC rats

Expressions of both the M2 and M3 muscarinic receptor subtypes in the bladder mucosa of PC rats were 2.17 (± 0.55 SE) and 1.87 (± 0.34) times higher than those in sham rats, respectively. Expressions of these receptor subtypes in the bladder detrusors of PC rats were similar to those in the sham rats (0.8 [± 0.10] times for M2; 0.98 [± 0.10] for M3). No statistically significant differences between the PC and sham groups were observed.

3.2 | In vitro relaxant effects by the combination of 5-HMT and mirabegron on bladder detrusor from PC rats

The relaxant effects of 5-HMT and mirabegron on EFS-induced contraction of bladder strips from PC rats showed that these drugs respectively inhibited 16.2% and 17.5% of the contractions induced by EFS. The concentration-relaxant response was 33.1% when the drugs were used in combination (Figure 1), demonstrating an additive relaxant effect ($P < 0.05$ vs individual treatments).

3.3 | Single cystometry

Cystometric parameters after vehicle administration were used as controls. BC and MV were significantly lower in PC rats compared with sham rats, but other parameters did not differ between groups (Figures 2 and 3).

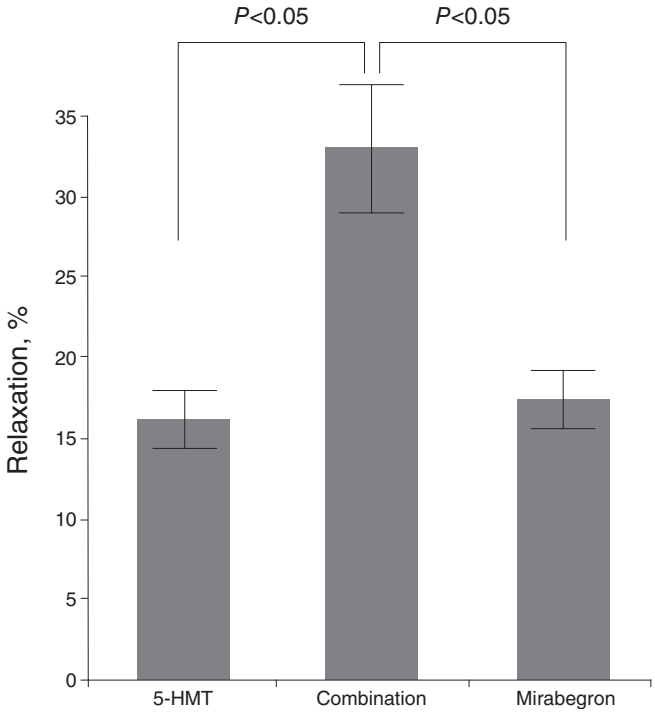


FIGURE 1 Relaxant effects by the single and combination of 5-HMT and mirabegron in electrical field stimulation-induced contraction of bladder detrusor. The analysis included six samples in each group. 5-HMT, 5-hydroxymethyl tolterodine

In PC rats, administration of 5-HMT or mirabegron led to slight and statistically insignificant increases in BC and decreases in MP. Residual volume did not change after administration of 5-HMT or mirabegron. However, combination treatment with both drugs increased BC (from 0.55 ± 0.02 mL to 0.93 ± 0.06 mL; $P < .01$) compared with either monotherapy group (Figure 4). Residual volume was also increased (from 0.02 ± 0.01 mL to 0.21 ± 0.10 mL; $P < 0.05$) after combination treatment, but there was no significant difference compared with either monotherapy group. The decrease in MP was greater in the combination group than in the monotherapy groups, but this difference was but not statistically significant. Other parameters did not change after combination treatment in comparison to before treatment.

4 | DISCUSSION

Our study found that expression of both M2 and M3 receptor subtypes in the mucosa was approximately twice as high in PC model rats as in sham-operated rats. Additive relaxant effects of 5-HMT and mirabegron were observed in the EFS-induced contractions of bladder strips from PC rats. Although in vivo experiments found that intravenous administration of low doses of 5-HMT or mirabegron did not influence any single cystometry parameters, combined treatment with these agents significantly increased BC in PC rats, suggesting a potential synergistic effect.

Our study used female PC rats, which showed frequent micturition and a decrease of BC. Pelvic venous congestion reduces blood flow to the bladder,[14] which is presumed to be one of the causal factors of idiopathic OAB.[15] Pelvic venous congestion might also induce hypoxia of the bladder. The expression of both M2 and M3 receptor subtypes in the urothelium were up-regulated in this model.

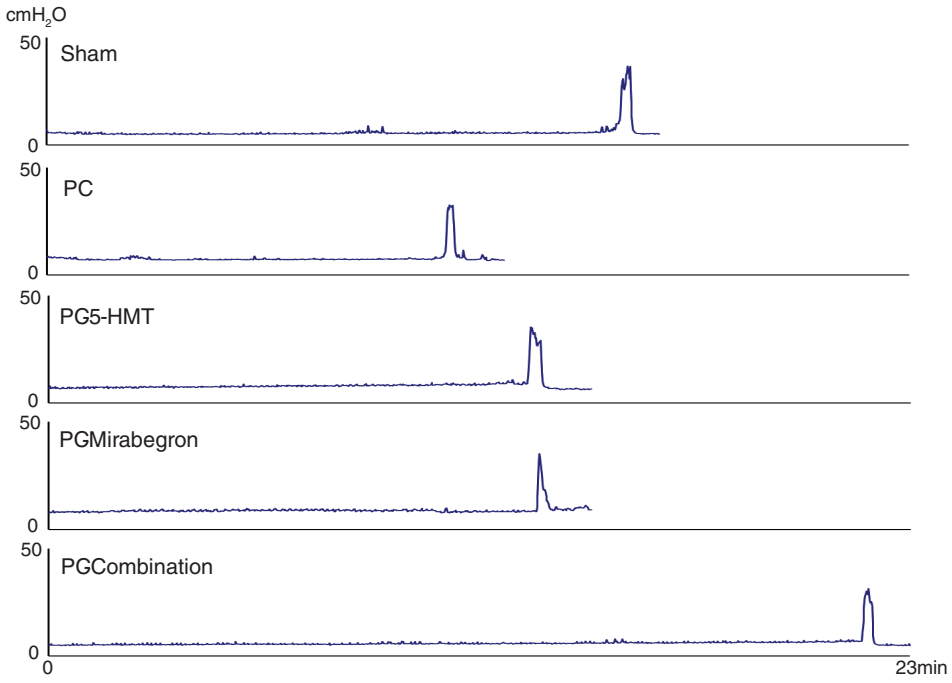
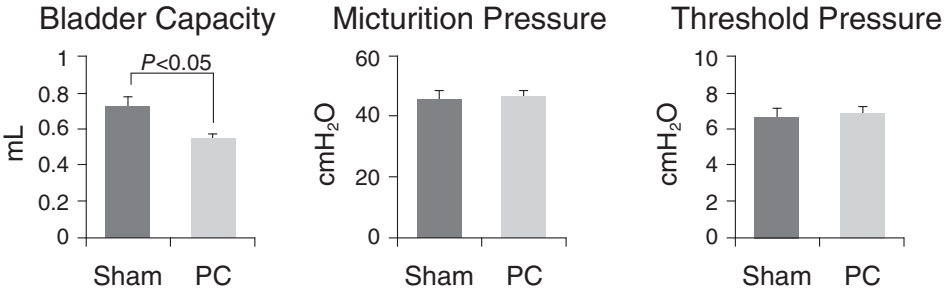
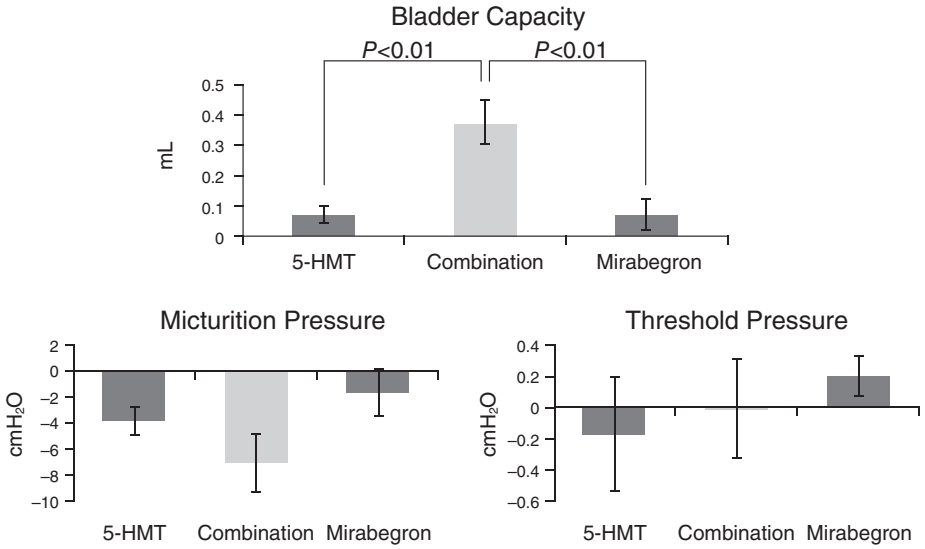


FIGURE 2 Representative traces of single cystometry in a sham rat, PC rat, PC rat with 5-HMT (PC-5-HMT), PC rat with mirabegron (PC-Mirabegron), and PC rat with combination of 5-HMT and mirabegron (PC-combination). 5-HMT, 5-hydroxymethyl tolterodine; PC, pelvic congestion [Color figure can be viewed at wileyonlinelibrary.com]



Variables	Sham Mean ± SE	PC Mean ± SE	Difference
Bladder capacity, mL	0.73±0.14	0.55±0.05	–0.18 (–24.7%)*
Micturition pressure, cmH_2O	45.27±8.51	46.33±6.59	1.06 (2.3%)
Threshold pressure, cmH_2O	6.64±1.54	6.89±0.88	0.25 (3.8%)
Residual volume, mL	0.01±0.00	0.01±0.00	0.00 (0%)

FIGURE 3 Cystometric study between PC rats and sham rats. PC, pelvic congestion. *P < 0.05; analysis of variance



Variables	5-HMT (n=8) Mean ± SE	Mirabegron (n=8) Mean ± SE	Combination (n=8) Mean ± SE	*P* Value vs Combination (Dunnett's test)
Bladder capacity, mL	0.07±0.03	0.07±0.05	0.37±0.07*	*P*<0.01: 5-HMT *P*<0.01: mirabegron
Micturition pressure, cmH_2O	–3.85±1.08	–1.65±1.77	–7.02±2.20	*P*>0.05: 5-HMT *P*>0.05: mirabegron
Threshold pressure, cmH_2O	–0.17±0.37	0.20±0.13	–0.01±0.32	*P*>0.05: 5-HMT *P*>0.05: mirabegron
Residual volume, mL	0.00±0.00	0.00±0.02	0.19±0.09	*P*>0.05: 5-HMT *P*>0.05: mirabegron

FIGURE 4 Mean changes of cystometric parameters. 5-HMT, 5-hydroxymethyl tolterodine; PC, pelvic congestion. *P < 0.01; change from baseline (analysis of variance)

Up-regulation of M2 and M3 has also been observed in other lower urinary tract symptoms models, including models of stroke and spinal cord injury,[16,17] as well as in humans with idiopathic detrusor overactivity.[18] In OAB, urinary frequency is developed along with reduction of functional BC, so the PC rat was suitable as an animal model of OAB. Because BC is a general metric by which the pharmacologic effects of drugs can be measured, BC was established as the primary endpoint in this in vivo study.

The detrusor strip contraction experiments detected an additive relaxant effect of 5-HMT and mirabegron on EFS-induced

contractions, which was likely caused by the independent effects of different mechanisms of action. 5-HMT and mirabegron might act independently on detrusor constrictions.

In vivo experiments found that the combination of 5-HMT and mirabegron demonstrated a potential synergistic effect on BC in PC rats. BC was increased by 0.37 mL under the combination therapy, which was greater than the sum of the increase following either monotherapy (0.07 mL each for 5-HMT and mirabegron). Expression of M2 was twice as high in PC model rats as in sham-operated rats. As MP and TP remained unchanged in these experiments, the increase in BC was not mediated by development of voiding dysfunction. In comparison, only an additive effect on detrusor constrictions was absorbed in vitro. The difference between the in vivo study and in vitro study results may be a result of the efferent pathway via urothelium. Changes in the urothelium and afferent and efferent nerves, as well as myogenic changes in the detrusor, are known to be main contributing factors to the pathology of OAB.[19] Afferent nerves, especially mechanosensitive Aδ- and C fibers, are known to be responsive to bladder contractions.[20] Recent reports have characterized the inhibitory effects of mirabegron on Aδ fibers by β_3 adrenoceptor stimulation[20] and the effect of 5-HMT on capsaicin-sensitive C fibers by antagonizing bladder muscarinic receptors.[21] Other antimuscarinics have also shown similar effects.[20,21] These studies suggest that the primary mechanism underlying the synergistic effects of combination therapy may be the inhibition of ACh by 5-HMT via the M2 receptor, which would restore primary activity of a β_3 agonist. In the case of the drugs used in this study, high expression of ACh and M2 might inhibit the activity of mirabegron by blocking AC in pathologic situations. The potential synergistic effects of combined administration of 5-HMT with mirabegron might therefore involve the restoration of mirabegron's effects by 5-HMT. A similar result was reported with the combination of a β_3 agonist (vibegron) and a nonselective antimuscarinic agent (tolterodine).[22] The increase in BC was greater when vibegron was coadministered with tolterodine compared to coadministration with darifenacin, a selective M3 antagonist, in rhesus monkeys.

Before fesoterodine and mirabegron can be used in combination in clinical practice, potential for pharmacokinetic drug-drug interactions should be investigated. For fesoterodine and mirabegron, this has been predicted based on well-established physiologically based pharmacokinetic models (Simcyp). The increase in the peak plasma concentration and the area under the plasma concentration-time curve of 5-HMT following concomitant administration of mirabegron and fesoterodine was <25%, which was not considered to be a clinically relevant drug-drug interaction for fesoterodine.[23]

Lastly, the potential for an overall increase in side effects from combining drug therapies utilizing different mechanisms of action must also be explored. Such side effects may be difficult to determine based on in vitro studies alone, given potential differences between antimuscarinic selectivity behavior with in vitro assays versus in vivo systems.[24] Evidence supports that fesoterodine and 5-HMT both bind bladder and detrusor tissue with higher affinity than the parotid gland competitively and reversibly,[25] indicating their selectivity for the target tissues. Another modeling and simulation study showed that when fesoterodine and mirabegron were coadministered, predicted changes in 5-HMT plasma concentrations were clinically insignificant, and did not warrant changes in the recommended daily dose of fesoterodine when given with mirabegron.[26] Notably, no differences were observed in side effects reported in a clinical trial by treatment groups receiving nonselective or selective antimuscarinics alongside a β_3 agonist.[27] Collectively, these studies suggest that combined use of nonselective antimuscarinics and β_3 agonist therapies is unlikely to significantly increase overall side effects.

In conclusion, this study demonstrated the potential of synergistic effects of combining fesoterodine and mirabegron in PC rats given subtherapeutic doses of 5-HMT and mirabegron. Combined therapies of fesoterodine and mirabegron may more effectively provide relief compared to monotherapies in clinical practice; however, further work is needed regarding the safety and efficacy of concurrent treatment.

ACKNOWLEDGMENTS

This study was sponsored by Pfizer Japan Inc; Pfizer employees participated in the analysis plan, data analysis, and manuscript preparation. Medical writing assistance was provided by Jill E. Kolesar, PhD, of Complete Healthcare Communications, LLC, (North Wales, PA, USA), a CHC Group company, and was funded by Pfizer Japan Inc.

DISCLOSURE

This study was sponsored by Pfizer Japan Inc. Pfizer employees participated in the analysis plan, data analysis, and manuscript preparation. Medical writing assistance was provided by Jill E. Kolesar, PhD, of Complete Healthcare Communications, LLC, (North Wales, PA, USA), a CHC Group company, and was funded by Pfizer Japan Inc. Hidetomi Yamagami was an employee of Pfizer Inc at the time of writing. Masanori Hizue is an employee of Pfizer Japan Inc. Yoshihiko Ito and Shizuo Yamada each have a financial interest and/or other relationships with Pfizer Japan Inc, Asahi Group Holdings, Co, Ltd, and Southern Knights' Laboratory Co Ltd. Kimio Sugaya, Saori Nishijima, and Katsumi Kadekawa have no competing interests regarding this manuscript.

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REFERENCES

1. Abrams P, Cardozo L, Fall M, et al. The standardisation of terminology of lower urinary tract function: report from the standardisation subcommittee of the international continence society. *Am J Obstet Gynecol*. 2002;187(1):116-126.

2. Andersson KE. Antimuscarinics for treatment of overactive bladder. *Lancet Neurol*. 2004;3(1):46-53.
3. Igawa Y, Aizawa N, Homma Y. Beta3-adrenoceptor agonists: possible role in the treatment of overactive bladder. *Korean J Urol*. 2010;51 (12):811-818.
4. Abrams P, Kelleher C, Staskin D, et al. Combination treatment with mirabegron and solifenacin in patients with overactive bladder: efficacy and safety results from a randomised, double-blind, dose-ranging, phase 2 study (symphony). *Eur Urol*. 2015;67(3):577-588.
5. Drake MJ, Chapple C, Esen AA, et al; BESIDE Study Investigators-Efficacy and safety of mirabegron add-on therapy to solifenacin in incontinent overactive bladder patients with an inadequate response to initial 4-week solifenacin monotherapy: a randomised double-blind multicentre phase 3B study (BESIDE). *Eur Urol*. 2016;70(1):136-145.
6. MacDiarmid S, Al-Shukri S, Barkin J, et al. Mirabegron as add-on treatment to solifenacin in patients with incontinent overactive bladder and an inadequate response to solifenacin monotherapy. *J Urol*. 2016;196(3):809-818.
7. Yamaguchi O, Kakizaki H, Homma Y, et al. Safety and efficacy of mirabegron as 'add-on' therapy in patients with overactive bladder treated with solifenacin: a post-marketing, open-label study in Japan (MILAI study). *BJU Int*. 2015;116(4):612-622.
8. Hegde SS, Eglen RM. Muscarinic receptor subtypes modulating smooth muscle contractility in the urinary bladder. *Life Sci*. 1999;64 (6-7):419-428.
9. Uchida H, Shishido K, Nomiya M, Yamaguchi O. Involvement of cyclic AMP-dependent and -independent mechanisms in the relaxation of rat detrusor muscle via beta-adrenoceptors. *Eur J Pharmacol*. 2005; 518(2-3):195-202.
10. Malhotra B, Gandelman K, Sachse R, Wood N, Michel MC. The design and development of fesoterodine as a prodrug of 5- hydroxymethyl tolterodine (5-HMT), the active metabolite of tolterodine. *Curr Med Chem*. 2009;16(33):4481-4489.
11. Ney P, Pandita RK, Newgreen DT, Breidenbach A, Stöhr T, Andersson KE. Pharmacological characterization of a novel investigational antimuscarinic drug, fesoterodine, in vitro and in vivo. *BJU Int*. 2008;101(8):1036-1042.
12. Sugaya K, Nishijima S, Kadekawa K, Ashitomi K, Ueda T, Yamamoto H. Pelvic venous congestion with castration causes chronic prostatitis in rats. *Int J Urol*. 2016;23(5):431-435.
13. Imamura T, Ogawa T, Minagawa T, et al. Combined treatment with a beta3 -adrenergic receptor agonist and a muscarinic receptor antagonist inhibits detrusor overactivity induced by cold stress in spontaneously hypertensive rats. *NeurourolUrodyn*. 2017;36(4):1026-1033.
14. Sugaya K, Nishijima S, Kadekawa K, et al. Pelvic venous congestion induces lower urinary tract dysfunction in rats. *Biomed Res*. 2018;39 (6):269-277.
15. Azadzoi KM, Tarcan T, Kozlowski R, Krane RJ, Siroky MB. Overactivity and structural changes in the chronically ischemic bladder. *J Urol*. 1999;162(5):1768-1778.
16. Braverman A, Legos J, Young W, Luthin G, Ruggieri M. M2 receptors in genito-urinary smooth muscle pathology. *Life Sci*. 1999;64(6-7):429-436.
17. Maruyama S, Kurosawa S, Takagi Y, et al. Urodynamics and bladder muscarinic receptors in rats with cerebral infarction and bladder outlet obstruction. *Neurosci Lett*. 2007;414(1):80-84.
18. Mukerji G, Yiangou Y, Grogono J, et al. Localization of M2 and M3 muscarinic receptors in human bladder disorders and their clinical correlations. *J Urol*. 2006;176(1):367-373.
19. Tyagi P. Pathophysiology of the urothelium and detrusor. *Can Urol Assoc J*. 2011;5(5 suppl 2):S128-S130.
20. Aizawa N, Homma Y, Igawa Y. Effects of L-arginine, mirabegron, and oxybutynin on the primary bladder afferent nerve activities synchronized with reflexic, rhythmic bladder contractions in the rat. *NeurourolUrodyn*. 2015;34(4):368-374.
21. Aizawa N, Ito H, Sugiyama R, et al. Selective inhibitory effect of imidafenacin and 5-hydroxymethyl tolterodine on capsaicin sensitive C fibers of the primary bladder mechanosensitive afferent nerves in the rat. *J Urol*. 2015;193(4):1423-1432.
22. Di Salvo J, Nagabukuro H, Wickham A, et al. Pharmacological characterization of a novel beta3 adrenergic agonist, vibegron: evaluation of anti-muscarinic receptor selectivity for optimal combination therapy for overactive bladder. *J Pharmacol Exp Ther*. 2016;360(2): 346-355.
23. Lin J, Goosen T, Tse S, et al. Physiologically based pharmacokinetic modeling & simulation using Simcyp to predict drug-drug interaction when mirabegron is co-administered with fesoterodine. Paper presented at: International Continence Society Conference; September 13-16, 2016; Tokyo.
24. Yamada S, Kuraoka S, Osano A, Ito Y. Characterization of bladder selectivity of antimuscarinic agents on the basis of in vivo drug-receptor binding. *Int Neurourol J*. 2012;16(3):107-115.
25. Yoshida A, Fuchihata Y, Kuraoka S, et al. Fesoterodine, its active metabolite, and tolterodine bind selectively to muscarinic receptors in human bladder mucosa and detrusor muscle. *Urology*. 2013;81(4):920 e921-920 e925.
26. Lin J, Goosen TC, Tse S, Yamagami H, Malhotra B. Physiologically based pharmacokinetic modeling suggests limited drug-drug interaction for Fesoterodine when Coadministered with Mirabegron. *J Clin Pharmacol*. 2019;59:1505-1518.
27. Yamaguchi O, Kakizaki H, Homma Y, et al. Long-term safety and efficacy of antimuscarinic add-on therapy in patients with overactive bladder who had a suboptimal response to mirabegron monotherapy: a multicenter, randomized study in Japan (MILAI II study). *Int J Urol*. 2019;26(3):342-352.

How to cite this article: Sugaya K, Yamagami H, Nishijima S, et al. Effects of combined treatment with fesoterodine and mirabegron in a pelvic congestion rat model: Results from in vitro and in vivo functional studies. *Lower Urinary Tract Symptoms*. 2020;12:173–179. https://doi.org/10.1111/luts.12296