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SYNERGISTIC EFFECT BY CO-ADMINISTRATION OF TAMSULOSIN AND SOLIFENACIN ON BLADDER ACTIVITY IN RATS

Hypothesis / aims of study
Alpha-1 adrenergic receptor antagonist is the first-line medications for the treatment of benign prostatic hyperplasia (BPH), and it sometimes improve both voiding disorders and collecting disorders. Co-administration of the alpha-1 receptor antagonist and the anti-muscarinic agent is more effective for controlling overactive bladder (OAB) in BPH patients. It is known that bladder epithelial cells express muscarinic receptors and alpha-1 adrenergic receptors and secrete both acetylcholine and adenosine triphosphate (ATP), while bladder sensory nerve endings express muscarinic and purinergic receptors. Recent studies have also shown that intravenous administration of alpha-1 receptor antagonist inhibits ATP release into the bladder lumen, and application of muscarinic agonist provokes ATP release from cultured bladder epithelial cells. These results suggest that both alpha-1 receptor antagonist and anti-muscarinic agent inhibit ATP secretion from the bladder epithelium, and this effect may be one of the reasons for improvement of collecting disorders by these agents. In the present study, therefore, we examined the synergistic effect by co-administration of ineffective low-dose alpha-1 receptor antagonist (tamsulosin hydrochloride) and anti-muscarinic agent (solifenacin succinate) on bladder activity after bladder stimulation in rats.

Materials and methods
Forty-eight female Sprague-Dawley rats were used in this study. Rats were divided into four group, which were a control group (n = 12), a tamsulosin group (n = 12), a solifenacin group (n = 12), and a co-administration group. Rats from the tamsulosin and solifenacin groups were infused with tamsulosin hydrochloride (0.5 μg/kg/h) or solifenacin succinate (20 μg/kg/h), and those from the co-administration group were infused with both tamsulosin hydrochloride (0.5 μg/kg/h) and solifenacin succinate (20 μg/kg/h) via subcutaneously implanted Alzet osmotic minipumps. These dosages were setup by confirming that bladder activity did not influence by independent administration. Rats from the control group were infused with distilled water by the same procedure. After 2 weeks of treatment, we performed 2 examinations as following; Study 1: Twenty-eight rats (7 from each group) were anesthetized with urethane and a small-bore catheter was inserted into the bladder through the urethra to perform continuous cystometry. Physiological saline was infused into the bladder (0.05 ml/min) via the catheter and bladder activity was monitored. After cystometry was done with physiological saline, the rats also underwent continuous cystometry with a 0.1% acetic acid solution. Cystometry was performed for at least 60 min with each solution, and the changes of bladder activity were recorded. Study 2: Other 20 rats (5 from each group) were taken out of the cage and placed on a clean board, and the spontaneously voided urine was collected from each rat, carefully. After that, the rats were anesthetized with isoflurane and 0.1% acetic acid solution (1 mL) was infused into the bladder for 10 min via a urethral catheter. The animals received a subcutaneous injection of 100 mg of cefazolin sodium hydrate to prevent urinary tract infection. Spontaneously voided urine was also collected at 4-6 hours (day 0) after recovery from isoflurane anaesthesia, and at 1-7 days after bladder stimulation. The urinary ATP level was measured, and was compared between before and after bladder stimulation in each group. Results are reported as the mean ± standard deviation (SD). Student's unpaired t-test was used for statistical analysis, and $p < 0.05$ was considered to indicate statistical significance.

Results
Study 1: During continuous cystometry with physiological saline, there were no significant differences of any of the cystometric parameters among the 4 groups. When cystometry was done with acetic acid, the maximum bladder contraction pressure was significantly higher (6-12%) than that obtained with physiological saline in all groups. The interval between bladder contractions was also significantly shorter (26-40%) than those during cystometry with physiological saline in control group, tamsulosin group, and solifenacin group. In the co-administration group, however, there was no significant difference of the interval of bladder contractions between before and after infusion of acetic acid solution. Study 2: Before the infusion of acetic acid into the bladder, the urinary ATP level (1-12 mol/mg Cre×E-10) did not differ among the 4 groups. After bladder stimulation, the urinary ATP level of the control group and the tamsulosin group showed a significant increase to 381 ± 642 mol/mg Cre×E-10 and 443 ± 467 mol/mg Cre×E-10 on day 0. The urinary ATP level of the solifenacin group and co-administration group also showed a significant increase to 204 ± 360 mol/mg Cre×E-10 and 219 ± 268 mol/mg Cre×E-10 on day 0, but their increase degrees were lower (about 1/2) than those in the control group and the tamsulosin group. The urinary ATP level of each group returned to baseline at 7 days.

Interpretation of results
Administration of tamsulosin and/or solifenacin without bladder stimulation did not influence the cystometric parameters and urinary ATP level, suggesting that the administered dosages of these drugs were not influence normal bladder function. During cystometry with bladder stimulation, each agent did not inhibit urinary frequency by bladder stimulation. However, co-administration of ineffective low-dose tamsulosin and solifenacin could inhibit urinary frequency by bladder stimulation. On the urinary ATP level, solifenacin and co-administration groups inhibited the increase of the urinary ATP level after bladder stimulation.

Concluding message
Co-administration of low-dose tamsulosin and solifenacin, which are ineffective in each agent, inhibit the afferent pathway from the bladder. The effect of solifenacin may be related to the inhibition of ATP release from bladder epithelium. Tamsulosin may also inhibit the afferent pathway with the other mechanism except ATP.

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Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Institutional animal care and use committee of the University of the Ryukyus**