393

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COMPARISON OF THE EFFECTS OF ANTIMUSCARINIC AGENTS ON AUTONOMIC NERVOUS SYSTEM, URINARY ATP LEVEL AND BLADDER ACTIVITY IN RATS.

Hypothesis / aims of study
Bladder smooth muscle cells undergo contraction when exposed to acetylcholine secreted from the parasympathetic nerve terminals, and the efficacy of antimuscarinic agents is usually assessed on the basis of blocking the micturition reflex. However, the bladder epithelial cells expresses adrenergic and the muscarinic receptors, these cells produce acetylcholine and adenosine triphosphate (ATP), and the afferent nerve terminals in the bladder have muscarinic and purinergic receptors. Moreover, muscarinic receptors are suggested to modulate ATP release from the bladder. There are several kinds of antimuscarinic agents with the singularity to various speciality. Antimuscarinic drugs are also known to act on the autonomic nervous system. In the present study, therefore, we compare the effects of 4 antimuscarinic agents (propiverine hydrochloride, imidafenacin, tolterodine, and oxybutynin chloride) on plasma monoamine level, blood pressure, urinary ATP level and bladder activity before and after bladder stimulation in rats.

Materials and methods
Ninety- five female Sprague-Dawley rats were used in this study. The rats were divided into five groups, which were a control group (n = 19), a propiverine group (n = 19), an imidafenacin group (n = 19), a tolterodine group (n = 19) and an oxybutynin group (n = 19). Rats from the propiverine group and tolterodine group were administered 5 mg of propiverine hydrochloride or 0.5 mg of tolterodine dissolved in distilled water (0.5 mL) in the morning using a fine catheter without anaesthesia. In the evening, these rats were also administered the same volume of only distilled water. Rats from the imidafenacin group and oxybutynin group were administered 12 μg of imidafenacin or 0.5 mg of oxybutynin hydrochloride dissolved in distilled water (0.5 mL) twice a day. These doses correspond to about 20 times the human dosage. Rats from the control group were administered the same volume of distilled water twice a day. After 2 weeks of treatment, we performed 3 examinations as following; Study 1: Thirty-five 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 35 rats (7 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 halothane 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 halothane anaesthesia, and at 1-7 days after bladder stimulation. During this period after infusion of the acetic acid solution into the bladder, administration of distilled water or drugs was continued once or twice a day. The urinary ATP level was measured, and was compared between before and after bladder stimulation in each group. Study 3: Remaining 25 rats (5 from each group) were determined the blood pressure by the tail-cuff method using a programmed sphygmomanometer. After blood pressure measurement, rats were anesthetized with urethane, and blood was withdrawn from vena cava to measure the plasma monoamine (adrenaline, noradrenaline, dopamine, and serotonin) levels. 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 cystometric parameters among the 5 groups. When cystometry was done with acetic acid, the interval between bladder contractions was significantly shorter and the maximum bladder contraction pressure was significantly higher in the control group than those during cystometry with physiological saline. However, in the other 4 groups, there were no significant differences of the cystometric parameters between before and after infusion of acetic acid solution. Study 2: Before infusion of acetic acid into the bladder, the urinary ATP level (1-5 mol/mg Cre×E-10) did not differ among the 5 groups. After bladder stimulation, the urinary ATP level of the control group and the tolterodine group showed a significant increase to 650 ± 288 mol/mg Cre×E-10 and 681 ± 714 mol/mg Cre×E-10 on day 0, and 59 ± 33 mol/mg Cre×E-10 and 129 ± 167 mol/mg Cre×E-10 on day 1. The urinary ATP levels of the imidafenacin, oxybutynin and propiverine groups also showed a significant increase to 281 ± 289 mol/mg Cre×E-10, 289 ± 397 mol/mg Cre×E-10 and 104 ± 99 mol/mg Cre×E-10 on day 0, and 8 ± 7 mol/mg Cre×E-10, 39 ± 55 mol/mg Cre×E-10 and 9 ± 6 mol/mg Cre×E-10 on day 1, but their increase degrees were lower than those in the control group. The urinary ATP level of each group returned to baseline at 7 days. Study 3: Noradrenaline and dopamine levels in the propiverine group were significantly higher (noradrenaline: 74% increase, dopamine: 103% increase) than those in the control group. However, the significant differences of plasma monoamine levels were not observed between control group and each other groups. There were no significant differences in blood pressure levels among 5 groups.

Interpretation of results
Administration of propiverine, imidafenacin, tolterodine and oxybutynin without bladder stimulation did not influence the cystometric parameters or the urinary ATP level, suggesting that the administered dosages of these drugs were not influence normal bladder function. During cystometry with bladder stimulation, all 4 agents prevented both urinary frequency and the increase of bladder contraction pressure. Propiverine, imidafenacin and oxybutynin inhibited the increase of the urinary ATP level after bladder stimulation, but not tolterodine. Therefore, the inhibitory effects of propiverine, imidafenacin and oxybutynin on the bladder function might be partly due to blocking the increase of ATP release from the bladder epithelium. However, since the present study did not show the effects of 4 agents on the afferent nerve terminals and the central nervous system, it is still not known why all 4 agents including tolterodine prevented bladder overactivity after bladder stimulation. Propiverine increased catecholamine level without change of the blood pressure. It is reported that propiverine improve urinary frequency as well as stress urinary incontinence. Therefore, high catecholamine level by propiverine may contribute to improve stress incontinence.

Concluding message

The inhibitory effects of propiverine, imidafenacin and oxybutynin but not tolterodine on bladder activity may be partly due to blocking the increase of ATP release from the bladder epithelium. An increase of the urinary ATP level can be used as a marker for activation of the bladder epithelium.

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Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
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