Hypothesis / aims of study
Several antimuscarinic drugs are clinically used, and several clinical trials of the antimuscarinic drugs for the treatment of overactive bladder showed the different efficacy and safety profiles among drugs. Therefore, the present study was performed to compare the effects of various antimuscarinic drugs (atropine, oxybutynin, propiverine, temiverine, vamicamide, tolterodine, imidafenacin, darifenacin, and solifenacin) on human isolated urinary bladder.

Study design, materials and methods
Human urinary bladders (78 male and 15 female) were obtained from patients undergoing radical cystectomy due to bladder carcinoma. Smooth muscle strips were dissected from the body of urinary bladders. Each smooth muscle strip was suspended in an organ bath filled with Krebs-Henseleit solution, and isometric tension development was recorded. The effects of various antimuscarinic drugs on the contractions induced by carbachol (CCh: 10^{-8} – 10^{-5} M), KCl (80 mM), CaCl2 (5 mM) and EFS (supramaximal voltage, 0.3 msec duration, 2.5 - 40 Hz and 3 sec train) were evaluated. Furthermore, using microdialysis method, microdialysis probe was inserted into the smooth muscle strip, and Ringer solution was perfused into the probe at a constant flow rate of 2.0 µl/min. Dialysate was collected during EFS every 10 min and the amount of acetylcholine (ACh) released in the dialysate was measured by HPLC with ECD. The effects of various antimuscarinic drugs on ACh release in human bladder were evaluated.

Results
CCh caused concentration-dependent contractions in human detrusor smooth muscles. Pretreatment with various antimuscarinic drugs caused rightward shift of the concentration-response curves for CCh, except for high concentrations (10^{-5} M) of oxybutynin, propiverine, temiverine, and solifenacin which caused decreases of about 20 - 40 % of the maximum contractions to CCh. The rank order of pA2 values was: darifenacin ≥ imidafenacin ≥ atropine = tolterodine ≥ oxybutynin ≥ vamicamide > propiverine = temiverine = solifenacin. High concentrations (10^{-6} M) of oxybutynin, propiverine, temiverine, and solifenacin significantly inhibited the KCl- and CaCl2-induced contractions. EFS caused frequency-dependent contractions of human detrusor smooth muscles, which were significantly inhibited by various drugs. In the presence of 10^{-6} M atropine, oxybutynin, propiverine, and temiverine (10^{-5} M) significantly inhibited the atropine-resistant part of the contractions. EFS caused significant increase in ACh release from cholinergic nerve endings in human detrusor smooth muscles, which were significantly inhibited in a concentration-dependent manner by oxybutynin, imidafenacin, and solifenacin.

Interpretation of results
The present data showed that antimuscarinic drugs have different effects on human bladder smooth muscles. The inhibitory effects on KCl- and CaCl2-induced contraction, and atropine-resistant part of contraction in EFS-induced contraction by oxybutynin, propiverine, temiverine, and solifenacin suggest that the drugs have calcium antagonistic action on bladder detrusor smooth muscle contraction, as well as antimuscarinic action. It has been reported that prejunctional M2 receptor subtype stimulates ACh release from cholinergic nerve ending, and that stimulation of prejunctional M2/M4 receptor subtypes inhibits acetylcholine release. In the present study, oxybutynin, imidafenacin, and solifenacin significantly inhibited ACh release induced by EFS. These drugs have relatively high affinity for M1 receptors, which may contribute to the inhibitory effect of ACh release from cholinergic nerve ending in human detrusor smooth muscles.

Concluding message
The present results demonstrate that pharmacological effects of various antimuscarinic drugs on human detrusor smooth muscles are different. In addition, several drugs may have calcium antagonistic action and prejunctional inhibiting actions of ACh release from cholinergic nerve endings. The present data may provide useful information for clinical use of these drugs in treatment for overactive bladder.

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HUMAN SUBJECTS:  This study was approved by the Ethics committee of Kumamoto University and followed the Declaration of Helsinki. Informed consent was obtained from the patients.