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DIFFERENTIAL GENE EXPRESSION OF CHOLINERGIC MUSCARINIC RECEPTOR SUBTYPES IN HUMAN PAROTID GLAND AND URINARY BLADDER DETRUSOR

<u>Hypothesis / aims of study</u>

Detrusor smooth muscle from various species contains a mixed population of M_2 and M_3 subtypes, with M_2 receptors being predominant. It has been suggested that the M_3 receptors in the human bladder cause a direct smooth-muscle contraction through phosphoinositide hydrolysis, whereas the physiologic role for the M_2 receptors is to oppose to smooth-muscle relaxation mediated by sympathetic β-adrenoceptors. Antimuscarinics usually produce significant clinical improvement in patients with detrusor overactivity and overactive bladder (OAB) syndrome. However, the clinical utility of antimuscarinic agents is limited by their lack of selectivity, responsible for the classic anticholinergic side effects of dry mouth, constipation, blurred vision, tachycardia, and the effects on cognitive function. However, receptor selectivity is not the only basis on which a drug may be 'uroselective'. Although M3 selective agents have the potential to eliminate some of these adverse effects, for example, it would appear that M3 receptors in the bladder detrusor are identical to tissues elsewhere in the body (e.g., salivary gland). From a clinical standpoint, it would seem important to find in relative terms the ratio between a drug dose required for a desired therapeutic action and the dose producing side effects. Thus, we examined the difference in gene expression of cholinergic muscarinic receptor subtypes between the salivary gland and the urinary bladder detrusor and how its expression changes in association with aging and bladder outlet obstruction.

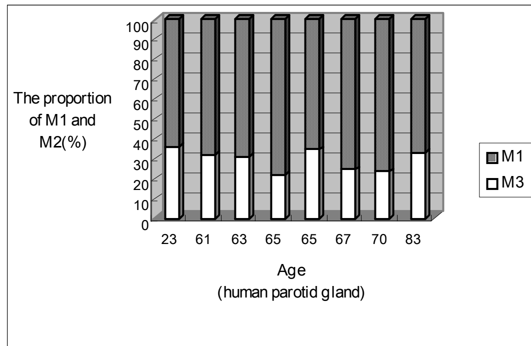
<u>Study design, materials and methods</u>

Human urinary bladder detrusor and parotid gland were obtained from 13 and 8 patients, respectively. The patients selected for urinary bladder detrusor samples did not have any lower urinary tract symptoms, as determined by International Prostate Symptom Score questionnaire. These specimens were obtained from macroscopically normal areas around the posterior wall of the bladder. To examine the effect of aging and obstruction in the rat, urinary bladder detrusor were taken from control bladder at the age of 7 and 13 weeks and obstructed bladder at the age of 13 weeks. The expression of mRNAs and proteins of muscarinic receptor subtypes were assessed by reverse transcription-polymerase chain reaction and Western blot analysis, respectively. The quantification of gene expression was assessed by a real-time RT-PCR with a Smart Cycler System (Cephied, Sunnyvale, CA, USA) by using SYBR green I as the fluorogenic dye (Molecular Probes, Eugene, OR, USA). The gene expression in each tissue was quantified as the product of the target gene relative to that of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. Statistical analyses were done using F tests and a two-tailed t-test (Microsoft Excel program). A p value of less than 0.05 was taken to indicate statistical significance.

<u>Results</u>

The RT-PCR and Western blot analysis showed the presence of M_1 (all samples), M_2 (2 of 8 samples), M_3 (all samples), M_5 (3 of 8 samples) subtypes in parotid gland, and M_2 (all samples), M_3 (all samples), M_5 (1 of 13 samples) subtypes in urinary bladder. However, M_4 subtype was not detected in both samples. The M_1 mRNA : M_3 mRNA ratio was 2.49 : 1.00 in human parotid gland(Fig1A), and the M_2 mRNA : M_3 mRNA ratio was 16.62 : 1.00 in human urinary bladder(Fig.1B). This expression ratio in human urinary detrusor was significantly different from each sample ($P<0.05$, F-tests), whereas that in human parotid gland was almost constant in each sample.

In the rat, M_1 and M_3 subtypes mRNAs were expressed in all submandibular gland, whereas M_2 and M_3 subtypes mRNAs were expressed in all urinary bladder detrusor. The expression of M_2 and M_3 subtypes mRNAs significantly increased (Fig2, $P<0.05$, two-tailed t-test) and decreased ($P<0.005$) in BOO model, respectively. Aging did not affect significantly the mRNA expression of muscarinic receptor subtypes in the detrusor.



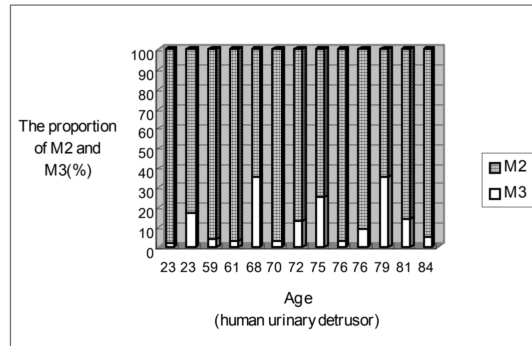


Fig1A Fig1B

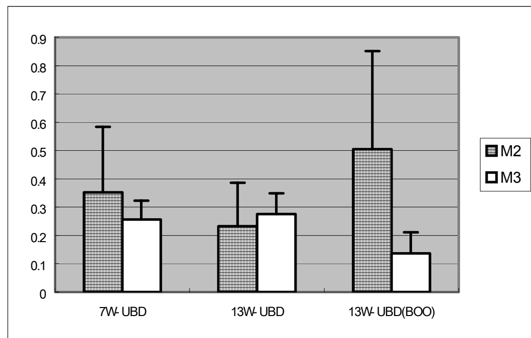


Fig2

UBD = urinary bladder detrusor, BOO = bladder outlet obstruction,
M = muscarinic receptor

Interpretation of results

Human urinary bladder muscle expressed muscarinic M_2 and M_3 subtype mRNA and proteins, and the proportion of these 2 subtypes were different significantly between individuals. On the other hand, the proportion of M_1 and M_3 subtype mRNA in human parotid gland were almost the same ratio. In the rat BOO model, the expression of M_3 and M2 subtype mRNA decreased and increased, respectively. Since the detrusor overactivity occurs in association with bladder outlet obstruction, this result may support the usefulness of non-selective antimuscarinics to overactive bladder symptoms in BOO patients.

Concluding message

Our results suggest that bladder outlet obstruction but not aging changes the proportion of expressed muscarinic receptor subtypes. The differential gene expression between individuals may support the usefulness of personalized medications in terms of subtype selectivity of antimuscarinic agents. When we could get the proportion of receptor subtypes expressed in the detrusor muscle, the subtype selectivity of the drug was chosen individually according to the proportion of its expression.

Reference

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