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BLADDER SELECTIVITY OF MUSCARINIC RECEPTOR BINDING OF FESOTERODINE, A NOVEL ANTIMUSCARINIC AGENT FOR TREATMENT OF OAB, IN HUMAN BLADDER DETRUSOR, MUCOSA AND PAROTID GLAND

Hypothesis / aims of study
Fesoterodine is a novel antimuscarinic agent for the treatment of overactive bladder (OAB). This agent is rapidly and extensively converted by non-specific esterases to its active metabolite, 5-hydroxymethyl tolterodine (5-HMT), which is also active metabolite of tolterodine [1]. Fesoterodine has similar affinity across five muscarinic receptor subtypes, whereas fesoterodine has lower potency than tolterodine and 5-HMT [2]. Mansfield et al. reported briefly equal binding affinity of fesoterodine for muscarinic receptors in human detrusor muscle and bladder mucosa homogenates [3].The current study aimed to characterize comparatively muscarinic receptor binding affinity of fesoterodine, 5-HMT and tolterodine in homogenates of human detrusor muscle, bladder mucosa and parotid gland.

Study design, materials and methods
Human urinary bladder specimens were collected from 8 patients undergoing total cystectomy for bladder carcinoma, and specimens of the human normal parotid gland were obtained from 4 patients undergoing the surgical excision of a parotid tumor with written informed consent obtained from all patients. None of the patients had diseases or used medications known to interfere with the cholinergic receptors system. The detrusor and mucosal tissues of the human bladder were separately cut into portions, and then stored at -80 °C until used. Also, all specimens of the human normal parotid gland were taken from areas macroscopically free of tumors and immediately frozen and stored -80 °C. Muscarinic receptors in the human tissue homogenates were measured by the radioligand binding assay using [^{3}H]NMS, and binding parameters of apparent dissociation constant (K_d) and maximal number of binding sites (B_{max}) for [^{3}H]NMS were estimated by nonlinear regression analysis. The inhibition constant, K_i, was calculated from the equation, $K_i=IC_{50}/(1+L/K_d)$, where IC_{50} and L represent the molar concentration of antimuscarinic agents necessary to displace 50 % of specific [^{3}H]NMS binding and the concentration of [^{3}H]NMS, respectively.

Results
Fesoterodine competed with [^{3}H]NMS for binding sites in human detrusor muscle, bladder mucosa and parotid gland in a concentration-dependent manner. The ratio of the parotid gland K_i value divided by both detrusor and bladder mucosa K_i value of each antimuscarinic agent was calculated as measure of bladder selectivity. Interestingly, muscarinic receptor binding affinity of fesoterodine was 20.5 and 7.3 times, respectively, higher in the detrusor muscle and bladder mucosa, respectively, than parotid gland. Similarly, 5-HMT and totelodine displayed 2.3-10.6 and 1.6-3.1, respectively, times higher affinity to muscarinic receptor in the human bladder over in the parotid gland (Table 1). Muscarinic receptor binding of fesoterodine was further characterized by measuring binding parameters of [^{3}H]NMS in the human detrusor muscle and parotid gland in the presence of fesoterodine at concentrations around the K_i value. Fesoterodine increased significantly K_d values for specific [^{3}H]NMS binding in the detrusor muscle (2.1 times) and parotid gland (3.0 times) compared with each control value, whereas the corresponding B_{max} values were little affected in the presence of fesoterodine (Table 2). Similarly, an increase of K_d was observed by 5-HMT and tolterodine.

Interpretation of results
The current results demonstrate that fesoterodine binds muscarinic receptors in human detrusor muscle and bladder mucosa with higher affinity than those in parotid gland. The in vitro bladder selectivity (estimated by ratios of K_i values between bladder and parotid gland) was the greatest for fesoterodine compared with 5-HMT and tolterodine. Further, these agents were shown to bind human muscarinic receptors in a competitive and reversible manner.

Concluding message
Fesoterodine binds human muscarinic receptors in human detrusor muscle and bladder mucosa with much higher affinity than those in parotid gland. This may contribute partly to the bladder selectivity of fesoterodine in patients with OAB receiving this agent.

Table 1. Competitive inhibition by fesoterodine, 5-HMT and tolterodine of specific [^{3}H]NMS binding in the human homogenates of detrusor muscle, bladder mucosa and parotid gland.

Tissues	Drugs	pK_i	nH
Detrusor muscle	Fesoterodine	7.98 ± 0.12 (20.5)	0.61 ± 0.06
	5-HMT	9.86 ± 0.03 (10.6)	0.64 ± 0.01
	Tolterodine	9.01 ± 0.12 (3.1)	0.95 ± 0.17
Bladder mucosa	Fesoterodine	7.51 ± 0.07 (7.3)	0.83 ± 0.05
	5-HMT	9.24 ± 0.13 (2.3)	1.04 ± 0.28
	Tolterodine	8.70 ± 0.06 (1.6)	0.88 ± 0.11

Parotid gland	Fesoterodine	6.65 ± 0.09 **	1.10 ± 0.17
	5-HMT	8.87 ± 0.11 *	0.87 ± 0.10
	Tolterodine	8.50 ± 0.07 *	1.18 ± 0.05

Each value represents the mean ± SEM for three to four experiments. Values in parentheses represent the ratio of parotid gland K_i values to the bladder detrusor or mucosa K_i. Asterisks show a significant difference from values in detrusor muscle, *P<0.05, **P<0.01.

Table 2. Effect of each antimuscarinic agent on binding parameter for specific [^{3}H]NMS binding in homogenates of human detrusor muscle and parotid gland.

Tissues	Drugs	K_d (pM)	B_{max} (fmol/mg protein)
Detrusor muscle	Control	201.6 ± 7.3	187.5 ± 25.0
	Fesoterodine (30 nM)	414.3 ± 21.6 (2.1)***	208.6 ± 19.0
	5-HMT (0.3 nM)	307.9 ± 50.5 (1.5)*	199.3 ± 31.8
	Tolterodine (3 nM)	531.0 ± 88.4 (2.6)**	173.6 ± 23.6
Parotid gland	Control	180.1 ± 10.2	171.6 ± 26.2
	Fesoterodine (300 nM)	534.5 ± 21.1 (3.0)***	176.2 ± 44.1
	5-HMT (3 nM)	861.4 ± 128 (4.8)***	182.5 ± 34.0
	Tolterodine (3 nM)	594.9 ± 69.3 (3.3)***	182.9 ± 38.7

Each value represents the mean ± SEM for four to six experiments. Values in parentheses represent the fold-increase in K_d values relative to control. Asterisks show a significant difference from each control value in detrusor muscle and parotid gland, *P<0.05, **P<0.01, ***P<0.001.

References

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