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COMPARISON OF RECEPTOR BINDING CHARACTERISTICS OF COMMONLY USED MUSCARINIC ANTAGONISTS IN HUMAN BLADDER DETRUSOR AND MUCOSA

Hypothesis / aims of study

Muscarinic receptor antagonists have long been the mainstay of therapy for patients with overactive bladder. Previously these antagonists were thought to block detrusor muscarinic receptors and inhibit bladder contraction. Surprisingly, clinical trials with the newer antagonists have demonstrated a beneficial effect on the symptom of urgency, a symptom not attributable to detrusor muscarinic receptor activation. Recent studies in humans (1) and animals (2) have indicated that muscarinic receptors are present on both mucosa and detrusor of the urinary bladder. These mucosal muscarinic receptors may represent a novel site of action of antimuscarinic agents.

Our primary aim was to examine the binding characteristics of three recently developed muscarinic receptor antagonists and compare them to oxybutynin, a widely used muscarinic receptor antagonist. The newer antagonists to be examined were darifenacin (M3 selective), trospium (non-selective) and fesoterodine (M3 preferring), which is currently under development. Our secondary aim was to compare the binding characteristics of these agents in the mucosa compared with the detrusor.

Study design, material and methods

Specimens of normal human detrusor (~2g) were collected from 10 control patients (8 males and 2 females) undergoing open bladder surgery for cancer procedures. Specimens were taken from macroscopically normal areas of the bladder. The bladder segment was dissected into mucosa (urothelium and lamina propria) and detrusor muscle and stored at –70^{o}C until use. All patients displayed normal micturition frequency, with no urge incontinence.

Radioligand binding with the muscarinic receptor ligand [^{3}H]quinuclidinyl benzylate (QNB) was performed using membranes from the detrusor muscle and mucosa (1). Membranes were prepared by homogenising the tissues in 50 mM sodium phosphate buffer. Increasing concentrations of oxybutynin, darifenacin, trospium and fesoterodine (10^{-12} M to 10^{-4} M) were coincubated with 200 pM [^{3}H]QNB for 2 hours at 37^{o}C. Incubations were terminated by filtration and washing with ice-cold buffer, through GF/B filters. Filter-bound radioactivity was quantified in a liquid scintillation counter.

Data were fitted to a one or two site model using the non-linear regression analysis program of GraphPad Prism (version 3). Dissociation constants of competitors for [^{3}H]QNB binding sites were calculated according to the formula $K_i = IC_{50} / (1+L/K_D)$, where IC_{50} denotes 50% inhibition of specific binding by the competitor, L is the concentration of radioligand, K_D is the dissociation constant of the radioligand. Previous saturation studies have determined that the K_D for [^{3}H]QNB in detrusor is 77.1 [55.2–99.0] pM and in mucosa the K_D is 100.5 [41.2–159.9] pM (1). Values are presented as K_i values (in nM) with 95% confidence limits.

Results

All antagonists displayed high affinity competition for [^{3}H]QNB binding to both detrusor and mucosa membranes. In detrusor, the order of potency was oxybutynin ≥ fesoterodine > trospium > darifenacin whereas in mucosa, the order was trospium > oxybutynin > fesoterodine > darifenacin. The data for darifenacin and fesoterodine were significantly better fitted to a two site rather than a one site model. Although oxybutynin exhitied a very low Hill slope, binding was not resolved into more than one site. Fesoterodine demonstrated high affinity binding (35% high affinity sites) in mucosal membranes but not in detrusor membranes. The high affinity component of darifenacin binding to mucosa and detrusor membranes represented 21% and 24% of total binding sites, respectively, with affinities corresponding to those reported for darifenacin binding to cloned human M3 receptors (cited

in ref. 1). The high affinity binding of darifenacin to mucosal membranes demonstrated affinity corresponding to that reported at either M3 or M5 receptors.

Table 1. Radioligand binding characteristics in bladder mucosa and detrusor membranes

Drug	n	Hill slope	Ki (95% CL)	Ki H (95% CL)*	% H
DETRUSOR					
Oxybutynin	5	0.31±0.03	0.85 (0.5-1.6)	N/A	
Fesoterodine	7	0.52±0.04	2.5 (1.8-3.3)	N/A	
Trospium	9	0.59±0.05	3.1 (2.2-4.3)	N/A	
Darifenacin	13	0.52±0.03	41.4 (32–52)	0.19 (0.08-0.47)	23±2
Drug	**n**	**Hill slope**	**Ki (95% CL)**	**Ki H (95% CL)***	**% H**
MUCOSA					
Oxybutynin	5	0.34±0.04	1.1 (0.67-1.9)	N/A	
Fesoterodine	5	0.51±0.03	2.7 (2.1-3.4)	0.09 (0.04-0.19)	35±2
Trospium	3	0.86±0.04	0.71 (0.62-0.81)	N/A	
Darifenacin	6	0.66±0.04	131 (106-162)	2.5 (0.7-0.9)	21±4

* data best fitted ($P<0.05$) to 2 site binding with high (H) and low (L) affinity components. N/A: two site binding was not preferred, therefore only the one site binding results are shown

Interpretation of results

One important finding of this study was that oxybutynin and fesoterodine were able to compete with equal affinity for [3H]QNB for binding to muscarinic receptors on both mucosa and detrusor membranes. However, trospium and darifenacin appeared to have different affinities in the two regions, although this finding would need confirmation. Receptor subtypes present in the human detrusor are 70% M2, 20% M3 and 10% M1, whereas in mucosa receptors were predominantly M2 with lower expression of M1, M3 and M5 (1). These results, together with the reported ability of muscarinic antagonists to influence the symptom of urgency, raise the possibility that mucosal muscarinic receptors may represent a novel site of action for muscarinic antagonists.

Concluding message

To our knowledge, this is the first demonstration that antimuscarinic agents currently being used to treat patients bind with high affinity to the muscarinic receptors in the mucosa as well as detrusor. This finding reinforces the concept that antimuscarinic drugs interact with receptors in the human urothelium and/or lamina propria as well as in detrusor.

References

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