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COMPARATIVE EVALUATION OF HUMAN MUCOSA AND DETRUSOR MUSCARINIC RECEPTOR BINDING BY ANTICHOLINERGIC AGENTS IN THE TREATMENT OF OVERACTIVE BLADDER

Hypothesis / aims of study

The urothelium is the epithelial lining of the urinary tract. Our traditional understanding of the function of this region was simply that of passive barrier between the urinary tract and its contents. In recent years, the urothelium exhibits neuron-like properties that contribute to sensory function. Although the function of such an innervation may be unclear, recent studies in humans and animals have indicated that muscarinic receptors (mAChRs) are present on both mucosa and detrusor of the urinary bladder [1, 2]. The mucosal mAChRs may represent a novel site of action of agents for the treatment of bladder disorders. Anticholinergic agents such as oxybutynin and propiverine are widely used for the treatment of overactive bladder. Tolterodine and darifenacin have been currently developed as novel anticholinergic agents that may exhibit pharmacological selectivity in the bladder. Furthermore, oxybutynin, propiverine and tolterodine are metabolized in the intestine and liver to form active metabolites, N-desethyl-oxybutynin (DEOB), 1-methyl-4-piperidyl benzilate N-oxide (DPr-P-4(N→O)) and 5-hydroxymethyl metabolite (5-HM), respectively. Although these metabolites are assumed to contribute to the mAChR blockade of parent compounds, their mAChR binding characteristics in the mucosa have not been examined. To clarify this issue, we have determined the affinity and density of mAChRs in both detrusor and mucosa of human urinary bladder, and then examined comparatively mAChR binding affinities of anticholinergic agents and their metabolites in these regions.

Study design, materials and methods

Specimens of human bladder were collected from bladder carcinoma patients (5-9 male) undergoing the open surgery. Specimens were taken from macroscopically normal areas of the bladder. The bladder segment was dissected into mucosa (urothelium and lamina propria) and detrusor, and stored at -80 until use. The mAChR in tissue homogenates was measured by radioreceptor binding assay with [N-methyl-^{3}H]scopolamine ([^{3}H]NMS) as a radioligand, and binding parameters of apparent dissociation constant (Kd) and maximal number of binding sites (Bmax) for [^{3}H]NMS were estimated by Scatchard analysis. The ability of anticholinergic agents and metabolites to inhibit specific [^{3}H]NMS binding was estimated from IC_{50} values, namely the molar concentrations of unlabeled drugs necessary to displace 50% of specific [^{3}H]NMS binding. Ki was calculated by using the equation, $Ki=IC_{50}/(1+l/Kd)$, where L is the concentration of [^{3}H]NMS (1 nM). The Ki values express the potency of anticholinergic agents in competing for [^{3}H]NMS binding sites in human tissues.

Results

Specific binding of [^{3}H]NMS (0.1 to 0.5 nM) in homogenates of human mucosa and detrusor increased the concentration–dependently, and it appeared to be saturable around 1 nM. Scatchard analysis revealed a liner plot in both regions, suggesting a single population of high affinity binding sites. The calculated Kd and Bmax values for specific [^{3}H]NMS binding in the mucosa were 260±82 pM and 69.8±4.3 fmol/mg protein, respectively. Similar values were obtained in the detrusor (Kd: 237±49 pM, Bmax: 85.3±5.7 fmol/mg protein). In the competition-binding experiments, anticholinergic agents and their metabolites competed with [^{3}H]NMS for the binding sites in human mucosa and detrusor in a concentration-dependent manner. Based on the Ki values, the mAChR binding affinities of oxybutynin, DEOB, propiverine, DPr-P-4(N→O), tolterodine and 5-HM were similar in both regions, while the affinity of darifenacin was 1.5 times lower in the mucosa than in the detrusor.

Interpretation of results

The major findings of this study are that 1) there may be a substantial population of mAChRs in the human urinary bladder mucosa, exhibiting similar affinity and density of mAChRs as those in the detrusor and 2) anticholinergic agents, widely used in the treatment of overactive bladder, are able to bind with equal affinity to mAChRs in human mucosa and detrusor. On the other hand, darifenacin appeared to have lower affinity for the mucosa compared with the detrusor, suggesting that the mucosa contains less population of M_3 and/or M_5 mAChRs [3].

Concluding message

The present study has provided that anticholinergic agents to treat patients with overactive bladder bind with high affinity to mAChRs in the mucosa as well as in the detrusor. This finding demonstrates that anticholinergic agents may interact with mAChRs not only in the human detrusor but in the urothelium and/or lamina propria. The identification of mAChR subtypes in the human mucosa is further under way.

References:
[1] Br J Pharmacol 129: 416-419, 2000.
[2] Br J Pharmacol 144: 1089-1099, 2005.
[3] J Urol 175: 365-369, 2006.

FUNDING: **NONE**
DISCLOSURES: **NONE**

HUMAN SUBJECTS: **This study was approved by the University of Shizuoka, Hamamatsu University School of Medicine and followed the Declaration of Helsinki Informed consent was obtained from the patients.**