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M3 SELECTIVE ANTIMUSCARINICS AFFECT GASTROINTESTINAL TRANSIT IN THE MOUSE MORE POTENTLY THAN NONSELECTIVE DRUGS

Hypothesis / aims of study

Antimuscarinic drugs are the mainstay of the treatment of overactive bladder (OAB). By their action on M_3 and/or M_2 receptors they interfere with the contractile action of acetylcholine (ACh) on the bladder smooth muscle. ACh is also an important mediator of intestinal function facilitating intestinal transit and content propulsion. Blockade of intestinal muscarinic receptors may cause a slowdown of transit and thus induce constipation.
The present study was designed to investigate the effect of the antimuscarinic drugs fesoterodine and tolterodine (non-selective) as well as darifenacin and solifenacin (M_3 selective) on intestinal function. Fesoterodine is currently in clinical testing for the treatment of OAB.

Study design, materials and methods

Radioligand binding experiments: Binding of fesoterodine, its metabolite SPM 7605 and tolterodine to muscarinic acetylcholine receptors was determined using membrane preparations of Chinese hamster ovary (CHO) cells expressing the different human muscarinic receptor subtypes (M_1-M_5). Incubations with the tritiated radioligands pirenzepine (2 nM, M_1), AF-DX384 (2 nM, M_2), 4-DAMP (0.2 nM, M_3-M_5) and the test substances (0.1 nM - 0.1 mM) were performed at 22°C for 60 minutes. The reaction was stopped by filtration and bound radioactivity was measured by scintillation counting. Non-specific binding was assessed by addition of atropine (1 µM). IC_{50} values and Hill coefficients (nH) were determined by non-linear regression analysis of the competition curves using Hill equation curve fitting. Inhibition constants (Ki) were calculated from the Cheng Prusoff equation.

Gastrointestinal transit: The method used is based on the distance travelled by a charcoal suspension given as test meal. After overnight fast, male Rj:NMRI mice (b.w. 18.5 - 23.2 g, n=8/group) were treated with fesoterodine, tolterodine, darifenacin, solifenacin (10 and 30 mg/kg p.o.) and atropine (20 mg/kg p.o., reference substance) in a volume of 10 mL/kg body weight. Sixty minutes later, a suspension of 10% activated charcoal and 2.5% arabic gum in distilled water (w/v; 0.4 mL/mouse) was administered orally. The animals were sacrificed 20 minutes later by cervical dislocation and the small intestine was removed from the cardia to the caecum. The distance covered by the charcoal (l) and the total length of the small intestine (L) were measured. Results are expressed as means ± SEM (of percent transit = l/L x 100) or as percent change from control. Student's t test was used for statistical analysis and $p<0.05$ was considered significant.

Results

Receptor binding studies:
The binding results at human recombinant M_1-M_5 receptors for fesoterodine, its metabolite SPM 7605 and tolterodine are presented in the table. For comparison, data for darifenacin and solifenacin from published studies have been added.

Binding of antimuscarinic substances to human muscarinic receptor subtypes

Muscarinic receptor subtype	pKi				
	Fesoterodine	SPM 7605	Tolterodine	Darifenacin[1]	Solifenacin[2]
M1	6.2	8.7	8.5	8.2	7.6
M2	6.3	8.8	8.2	7.4	6.9
M3	<6	8.2	7.9	9.1	8.0
M4	6.8	9.0	8.7	7.3	-
M5	<6	8.3	8.3	8.0	-

Muscarinic receptor subtype	pKi				
	Fesoterodine	SPM 7605	Tolterodine	Darifenacin[1]	Solifenacin[2]

Each determination was performed in duplicate.

Gastrointestinal transit

Under control conditions, 51 ± 3% of the small intestines of the mice were covered by charcoal. Atropine (20 mg/kg) significantly inhibited gastrointestinal transit by 31% to 35 ± 2% ($p<0.001$). Whereas fesoterodine and tolterodine had no significant effect (inhibition of transit at 30 mg/kg by 4% and 6%; $p>0.05$), darifenacin and solifenacin inhibited transit by 18% (ns) and 25% ($p<0.01$), respectively, at 10 mg/kg and by 29% ($p<0.01$) and 20% ($p<0.05$), respectively, at 30 mg/kg.

Interpretation of results

Fesoterodine, SPM 7605 and tolterodine are non-selective antimuscarinic drugs. SPM 7605 is more potent than its parent compound fesoterodine and can thus be regarded as the main active pharmacological principle of fesoterodine. SPM 7605 appears to be slightly more active than tolterodine at M_1-M_4 receptors. All three drugs show slightly higher affinity for the M_2 as compared to the M_3 receptor. In contrast, darifenacin and solifenacin have been reported to be 60-times and 12-times, respectively, more selective for the M_3 in comparison to the M_2 receptor.

The inhibition of intestinal transit in mice observed with darifenacin and solifenacin but not with fesoterodine and tolterodine suggests that antimuscarinic drugs with selectivity for M_3 receptors have a higher potential for impairing intestinal function than non-selective antagonists.

Concluding message

Salivary secretion and intestinal motility are attenuated by antimuscarinic drugs, and thus dry mouth and constipation are frequently observed side effects in clinical trials. The data presented here indicate that M_3 selective compounds have a stronger influence on intestinal function in comparison to non-selective antimuscarinic drugs in this mouse model. Whether similar effects are observed in man has to be investigated and verified in clinical trials.

References

(1) Darifenacin: a novel M_3 muscarinic selective receptor antagonist for the treatment of overactive bladder. Expert Opin Investig Drugs 2004;13:1493-1500

(2) M_3 receptor antagonism by the novel antimuscarinic agent solifenacin in the urinary bladder and salivary gland. Naunyn Schmiedebergs Arch Pharmacol 2002;366:97-103