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DARIFENACIN IS SELECTIVE FOR THE HUMAN RECOMBINANT M_3 RECEPTOR SUBTYPE

Aims of Study

The M_3 receptor is the primary receptor in the pathogenesis of overactive bladder (OAB) syndrome, making it an attractive target for treatment intervention. The clinical effectiveness of current treatments (oxybutynin, tolterodine, propiverine and trospium) may be limited by their lack of M_3 receptor selectivity. Darifenacin is the first selective M_3 muscarinic antagonist under clinical evaluation for the treatment of this common but under-reported condition.

The objective of this study was to compare the binding affinity of darifenacin for the human recombinant muscarinic receptor subtypes (M_1-M_5) with four antimuscarinic drugs (tolterodine, oxybutynin, propiverine and trospium) used in the management of OAB.

Methods

The binding affinities of test compounds were determined using CHO-K1 cell lines stably expressing M_1-M_5 receptors. Experiments were conducted at 20°C in HEPES buffer (20 mM, pH 7.4) using [N-methyl-^{3}H]-scopolamine (0.1-0.4 nM).

The binding affinities of test compounds were determined from competition experiments using 12 concentrations of antagonist. Non-specific binding was defined using 1 µM atropine. IC_{50} values were obtained from competition curves using an in-house data fitting programme. K_i values were derived from IC_{50} values using the Cheng-Prusoff IC_{50} correction (1). Data were compared for M_3 selectivity using analysis of variance (ANOVA).

Results

Data are expressed as mean values ± standard error of the mean (SEM) of *n* experiments. The affinity of antimuscarinic compounds for the human recombinant receptor subtypes M_1-M_5 are shown in Table 1.

Table 1. Affinity (pK_i) of antimuscarinic compounds for the human recombinant receptor subtypes M_1-M_5

pK_i	M_1	M_2	M_3	M_4	M_5
Darifenacin	8.2(0.04)	7.4(0.1)	9.1(0.1)	7.3(0.1)	8.0(0.1)
Tolterodine	8.8(0.01)	8.0(0.1)	8.5(0.1)	7.7(0.1)	7.7(0.03)
Oxybutynin	8.7(0.04)	7.8(0.1)	8.9(0.1)	8.0(0.04)	7.4(0.03)
Propiverine	6.6(0.1)	5.4(0.1)	6.4(0.1)	6.0(0.1)	6.5(0.1)
Trospium	9.1(0.1)	9.2(0.1)	9.3(0.1)	9.0(0.1)	8.6(0.1)

pK_i data presented as mean (SEM) (n = 3-6).

A comparison of the M_3 selectivity of each compound (ie, ratio of mean binding affinity at each receptor subtype) is shown in Table 2.

Table 2. Comparison of the M_3 selectivity of each compound

	M_3 vs M_1	M_3 vs M_2	M_3 vs M_4	M_3 vs M_5
Darifenacin	9.3**	59.2**	59.2**	12.2**
Tolterodine	0.6*[(b)]	3.6**	7.3**	6.3**
Oxybutynin	1.5*[(a)]	12.3**	6.9**	27.0**
Propiverine	0.6*[(b)]	9.6**	2.8**	0.8
Trospium	1.5	1.3	2.0*[(a)]	4.6**

The ratio of the K_i values were derived from the antilog of the difference in the mean pK_i values shown in Table1.
K_i ratios were compared by ANOVA. *p<0.05, **p<0.001.
[(a)] although statistically significant unlikely to be biologically relevant.
[(b)] statistically significant selectivity for M_1 although unlikely to be biologically relevant.

Conclusions

Darifenacin showed the greatest selectivity for the M_3 receptor over other muscarinic receptor subtypes. In contrast, the antimuscarinic agents currently in use for OAB did not demonstrate obvious M_3 selectivity. This distinct property may confer improved clinical effectiveness for darifenacin over standard treatments for OAB.

References

1. Cheng Y, Prusoff WH. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (IC_{I50}) of an enzymatic reaction. *Biochem Pharmacol* 1973; 22(23): 3099-3108.