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DISCOVERY & DEVELOPMENT OF SELECTIVE M_3 ANTAGONISTS FOR CLINICAL USE

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Summary

The treatment of airway obstructive disease may be improved by antimuscarinic agents which selectively block M_1 and M_3 receptors but do not inhibit prejunctional cholinergic autoreceptors which limit release of acetylcholine. Revatropate is a novel antimuscarinic agent which shows some 50-fold selectivity for M_1 and M_3 receptors in guinea pig trachea and rabbit vas deferens over the M_2 subtype in atria. This selectivity profile was seen *in vivo* in anaesthetised guinea pigs and conscious dogs where bronchodilator activity was produced in the absence of any effect on heart rate. Revatropate, in contrast to the non-selective agent ipratropium, did not potentiate bronchoconstrictor responses induced by vagal nerve stimulation, indicating that inhibitory autoreceptors were still functional. Early clinical studies in COAD patients showed that inhaled revatropate was an effective bronchodilator which was well tolerated. Darifenacin differs from revatropate by showing selectivity for M_3 receptors relative to both M_2 and M_1 subtypes. [^{3}H] darifenacin had 5-fold higher affinity for the human m_3 relative to m_1 receptors while there was significantly reduced binding to m_2, m_4 and m_5 receptors. The degree of selectivity in functional tissue preparations was even greater, with darifenacin showing 100-fold selectivity for the ileum M_3 receptors over M_2 receptors in atria and 30-fold over M_1 receptors in rabbit vas deferens. Darifenacin was able to differentiate between M_3 receptors in different tissues; although darifenacin was equipotent with atropine in the ileum and bladder, it was some 10-fold and 6-fold less potent at inhibiting muscarinic responses in the trachea and submandibular salivary gland respectively, relative to atropine. Studies in anaesthetised dogs confirmed this selectivity profile. Thus darifenacin inhibited responses of the gut and bladder to cholinergic stimulation without affecting heart rate. Salivary gland responses were inhibited at doses some 6-10 fold higher than those required to inhibit gut and bladder responses. Clinical studies are ongoing in urge incontinence and functional bowel disease which may confirm this selectivity profile.

Key Words: **muscarinic receptor subtypes, M_3 antagonists, preclinical pharmacology**

The Datura genus of plants which contain atropine and related anticholinergic alkaloids, have been used by man for several thousand years for a variety of medical and other purposes. Thus for example, inhalation of smoke from the herb Datura stramonium was recommended for the treatment of asthma in the seventeenth century (1). However, drying of secretions, tachycardia, urinary retention, blurred vision and central nervous system effects were all recognised complications associated with the use of atropine containing compounds and preparations. Muscarinic antagonists are still widely used, for example oxybutynin in urinary urge incontinence and dicyclomine in irritable bowel syndrome, but the clinical utility of these non-selective agents is still limited by adverse side effects.

In 1976, Barlow's studies of the relative activities of a series of muscarinic antagonists in the guinea pig ileum and the rabbit atrium indicated that responses in these two tissues were mediated by different receptors (2). Further work clearly established the heterogeneity of muscarinic receptors and they have been classified using pharmacological techniques into M_1, M_2, M_3 and M_4 subtypes, and on genomic cloning into m1, m2, m3, m4 and m5 subtypes (3). Thus the possibility of designing sub-type selective agents to achieve efficacy with reduced side-effects was a feasible and exciting possibility. Muscarinic M_3 receptors are located predominantly on smooth muscle and salivary glands, and it was considered that agents selective for this sub-class of receptors could have therapeutic utility in the treatment of incontinence, disorders of gastro-intestinal motility and as bronchodilators in respiratory disease. We therefore initiated projects at Pfizer to identify M_3 selective antagonists and this paper describes the profile of 2 such agents, revatropate (UK-112,166) and darifenacin (UK-88,525) (Fig. 1).

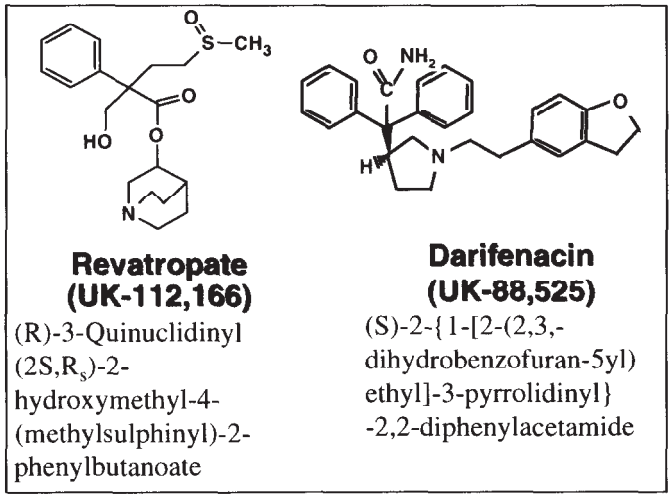

Fig. 1
Structural formulae of novel M_3 antagonists.

Revatropate: - a drug for treatment of COAD

Activation of cholinergic nerves is the major bronchoconstrictor neural mechanism in animal and human airways and there is evidence that this mechanism may be overactive in

muscarinic receptors M_1, M_2, and M_3 have been identified in human airways and their location and possible physiological function have been extensively reviewed (5). Stimulation of the vagus nerve releases acetylcloline (ACh) which activates muscarinic receptors on airway smooth muscle (M_3) and submucosal gland (M_3) to induce bronchoconstriction and mucus secretion respectively. M_1-receptors facilitate neurotransmission through parasympathetic ganglia and possibly enhance cholinergic reflexes. Additionally, there are also autoreceptors on cholinergic nerve terminals innervating bronchial and tracheal smooth muscle which inhibit ACh output when activated (6, 7) which have been characterised as the M_2 muscarinic subtype (5, 8). (In the guinea pig, there is some evidence that these autoreceptors may be more M_4-like rather than M_2 (9)). Thus a rational approach to producing an improved agent to treat airways obstruction was to identify a compound which selectively inhibited M_3 and M_1 muscarinic receptors, but unlike non-selective agents such as ipratropium, did not interfere with the neurotransmitter negative feedback loop by blockade of M_2 (or M_4)-receptors.

The *in vitro* profile of revatropate is shown in Table I. Revatropate showed potent antagonism of M_1 and M_3 receptors with some 50-fold selectivity over the M_2 subtype. This was in contrast to ipratropium which was also potent, but non-selective (10).

TABLE I

In vitro profile of revatropate and ipratropium.

TISSUE/SUBTYPE	REVATROPATE			IPRATROPIUM		
	pA_2 (95% conf. limits)	slope	n	pA_2 (95% conf. limits)	slope	n
Rabbit vas deferens M_1	9.26 (8.93-9.93)	1.06	11	9.44 (9.16-9.86)	1.11	14
Guinea pig atria M_2	7.25 (7.12-7.39)	1.19	16	9.15 (8.91-9.45)	1.21	4
Guinea pig trachea M_3	8.92 (8.67-9.24)	0.97	17	9.45 (9.24-9.73)	1.38	12

n=number of tissues

This selectivity profile was also seen *in vivo*. Thus in anaesthetised guinea pigs, revatropate antagonised ACh-induced M_3 mediated bronchoconstrictor responses at some 80-fold lower doses than those required to inhibit ACh-induced M_2 mediated bradycardia (10). Ipratropium inhibited both responses over a similar dose range.

The functional involvement of pre-junctional muscarinic receptors on post-ganglionic airway cholinergic nerves that inhibit the release of ACh in lung responses to nerve stimulation has been demonstrated in animal experiments (5, 8, 11). For example, bronchoconstrictor responses to vagal nerve stimulation were potentiated by the M_2/M_4-selective antagonist methoctramine, indicating inhibition of the inhibitory feedback control on ACh release. Similar experiments were carried out in our laboratories in anaesthetised guinea pigs and cats with revatropate and ipratropium, comparing their effect on bronchoconstriction induced by vagal stimulation with responses to injected ACh (12). Ipratropium potently inhibited i.v. ACh-induced bronchoconstriction in both species (ID_{50} 1.45μg/kg in guinea pig, 0.08μg/kg in cat). Significantly higher doses were required to inhibit responses to vagal nerve

stimulation (ID_{50}>10μg/kg in guinea pig, 2.1μg/kg in cat). Additionally, low doses of ipratropium potentiated the bronchoconstriction responses to vagal-nerve stimulation by up to 150%. (See Fig 2). Revatropate also potently inhibited the bronchoconstrictor response to i.v. ACh (ID_{50} 0.94μg/kg in guinea pig and 0.52 μg/kg in cat). However, in contrast to ipratropium, responses to vagal stimulation were similarly inhibited over the same dose range (ID_{50} 1.7μg/kg in guinea pig, 1.03μg/kg in cat). Potentiation of the vagal response was not observed at any dose. The data from guinea pigs are shown in Fig 2.

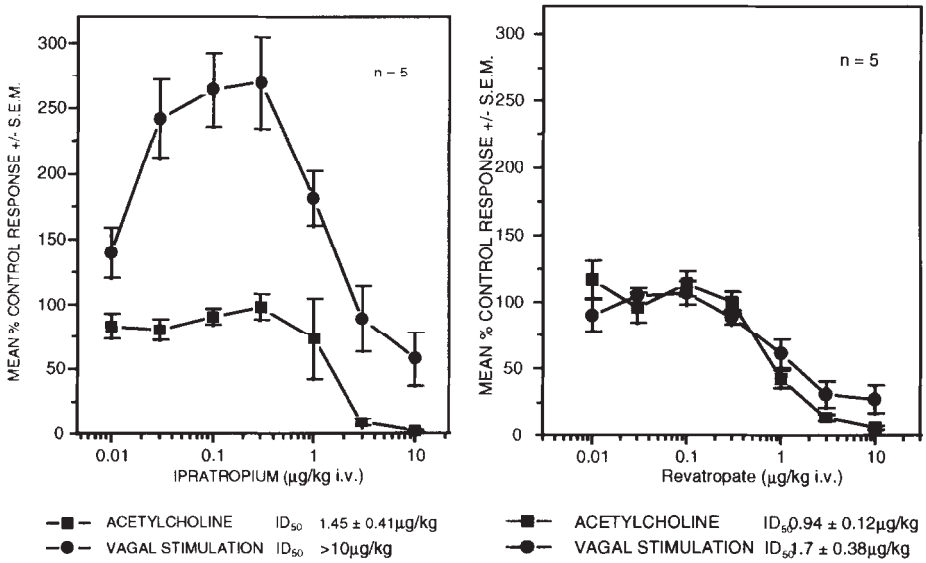

Fig. 2
Selectivity of revatropate for post-junctional M_3 receptors over pre-junctional autoreceptors - comparison with ipratropium in anaesthetised guinea pigs.

The bronchodilator activity and lung selectivity of revatropate was confirmed in conscious dogs where revatropate, given orally and by metered-dose inhaler, antagonised nebulised methacholine-induced bronchospasm without affecting heart rate (13).

The role of the autoreceptor on pre-junctional cholinergic nerve terminals in the airways in man is unknown, but indirect clinical pharmacology studies indicate their presence in non-asthmatics but absence in asthmatic subjects (14). It is possible, that while neuronal M_2 receptors exert an important inhibitory control on the parasympathetic nerves supplying the airway smooth muscle in normal circumstances, the situation may be different in the disease state. Thus measurements of vagal-induced bronchoconstriction responses in guinea pig indicated that prejunctional autoreceptors are dysfunctional after exposure to viral airway infections, ozone or antigen inhalation (15). Inflammatory mediators and/or eosinophil degranulation produces were implicated in the loss of M_2 function in these studies.

The first clinical study evaluating the effect of revatropate in COAD patients was very encouraging. Forty two COAD patients were studied in a double-blind 3 treatment cross-over study, designed to compare the response to 320μg revatropate, 80μg ipratropium bromide and

placebo given via Meter Dose Inhaler (16). The mean increase from pre-dose FEV_1 to peak-post dose FEV, was 0.34L (+27%) on revatropate, 0.36L (+29%) on ipratropium bromide and 0.15L (+12%) on placebo. Revatropate was well tolerated and no side effects were reported. Further studies are required to explore the dose-response relationships, and these may reveal advantages over non-selective agents.

Darifenacin

The functional smooth muscle responses induced by cholinergic nerve stimulation to the bladder and gut are mediated via M_3 receptor activation (3). Darifenacin differs from revatropate by showing selectivity for the M_3 subtype relative to all other muscarinic receptor subtypes and hence is anticipated to have an advantage over non-selective anti-muscarinic agents in the treatment of urge incontinence and functional bowel disease.

The binding of [^{3}H]-darifenacin to the five cloned human muscarinic receptors (m1 - m5) expressed in CHO cells was compared (17). [^{3}H]-darifenacin was found to bind with 5-fold higher affinity to m3 (K_D, 0.33nM) over m1 (K_D, 1.6nM) receptors. There was no specific binding to m2 receptors and specific binding to m4 and m5 receptors was insufficient to determine a K_D. Competition studies in cells expressing m3 and m1 receptors using a range of muscarinic antagonists showed that darifenacin represents the first selective m3 ligand. A greater degree of selectivity for the M_3 receptor subtype was observed in functional *in vitro* studies. The pA_2 values for darifenacin and atropine versus muscarinic activation in a variety of tissue preparations are shown in Table II. Atropine was essentially non-selective while darifenacin showed 100-fold selectivity for ileum M_3 receptors over M_2 receptors in atria and 30-fold over M_1 receptors in rabbit vas deferens (18, 19).

TABLE II

In vitro profile of darifenacin and atropine.

TISSUE/SUBTYPE	DARIFENACIN			ATROPINE		
	pA_2± sem	slope	n	pA_2 ± sem	slope	n
Guinea pig ileum M_3	9.44 ± 0.07	1.16	6	9.4 ± 0.07	0.96	6
Guinea pig trachea M_3	8.70 ± 0.09	1.08	6	9.20 ± 0.1	1.18	6
Guinea pig bladder M_3	8.66 ± 0.14	1.01	4	9.01 ± 0.09	0.86	4
Guinea pig atria M_2	7.48 ± 0.13	0.84	6	8.72 ± 0.06	1.13	7
Rabbit vas deferens M_1	7.90 ± 0.08	0.94	6	9.58 ± 0.09	0.9	7
Guinea pig salivary gland M_3	7.0 ± 0.1 (pIC_{50})	-	4	7.87 ± 0.14 (pIC_{50})	-	4

Functional responses to cholinergic activation of salivary glands are also mediated by the M_3 muscarinic subtype. However, although darifenacin was equipotent with atropine in the ileum and bladder preparations, darifenacin was some 6-fold less potent at inhibiting carbachol-induced ^{86}Rb efflux from the submandibular salivary gland and 10-fold less potent at

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