REVIEW

Muscarinic receptor antagonists, from folklore to pharmacology; finding drugs that actually work in asthma and COPD

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In the lungs, parasympathetic nerves provide the dominant control of airway smooth muscle with release of acetylcholine onto M3 muscarinic receptors. Treatment of airway disease with anticholinergic drugs that block muscarinic receptors began over 2000 years ago. Pharmacologic data all indicated that antimuscarinic drugs should be highly effective in asthma but clinical results were mixed. Thus, with the discovery of effective β-adrenergic receptor agonists the use of muscarinic antagonists declined. Lack of effectiveness of muscarinic antagonists is due to a variety of factors including unwanted side effects (ranging from dry mouth to coma) and the discovery of additional muscarinic receptor subtypes in the lungs with sometimes competing effects. Perhaps the most important problem is ineffective dosing due to poorly understood differences between routes of administration and no effective way of testing whether antagonists block receptors stimulated physiologically by acetylcholine. Newer muscarinic receptor antagonists are being developed that address the problems of side effects and receptor selectivity that appear to be quite promising in the treatment of asthma and chronic obstructive pulmonary disease.

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Abbreviations

COPD, chronic obstructive pulmonary disease; IUPHAR, International Union of Basic and Clinical Pharmacology; LD_{50}, lethal dose for 50% of population; M1–M5, muscarinic receptor subtypes 1–5

Muscarinic receptors in the lung

In the lungs, anticholinergic compounds block muscarinic receptors on airway smooth muscle, glands and nerves to prevent muscle contraction, gland secretion and enhance neurotransmitter release. There are five muscarinic receptor subtypes [designated M1 through M5 by the IUPHAR (Caulfield and Birdsall, 1998)] all belonging to the large family of seven transmembrane G-protein coupled receptors. In human lung (and in all animal species tested), acetylcholine induces bronchoconstriction by stimulating M3 (Figure 1) receptors on smooth muscle (Roffel *et al.*, 1990). Although airway smooth muscle contraction is mediated by M3 receptors, the majority of muscarinic receptors on airway smooth muscle are actually M2 (Barnes, 1993). These M2 receptors contribute indirectly to airway smooth muscle con-

traction by limiting β-adrenoceptor-mediated relaxation through inhibition of adenylate cyclase (Fernandes *et al.*, 1992). Glandular secretion is also mediated predominantly by M3 muscarinic receptors on submucosal cells (Marin *et al.*, 1976; Borson *et al.*, 1980; Phillips *et al.*, 2002).

Muscarinic receptors are also present on parasympathetic nerves supplying the lungs (Fryer and Maclagan, 1984). M2 muscarinic receptors on postganglionic parasympathetic nerves (Faulkner *et al.*, 1986; Fryer *et al.*, 1996) limit acetylcholine release, thus providing a physiologically relevant, negative feedback control over acetylcholine release (Fryer and Maclagan, 1984; Baker *et al.*, 1992). Blocking M2 receptors with muscarinic antagonists including atropine and ipratropium or using selective M2 receptor antagonists such as gallamine, significantly potentiates vagally induced bronchoconstriction (Fryer and Maclagan, 1984; 1987; Blaber
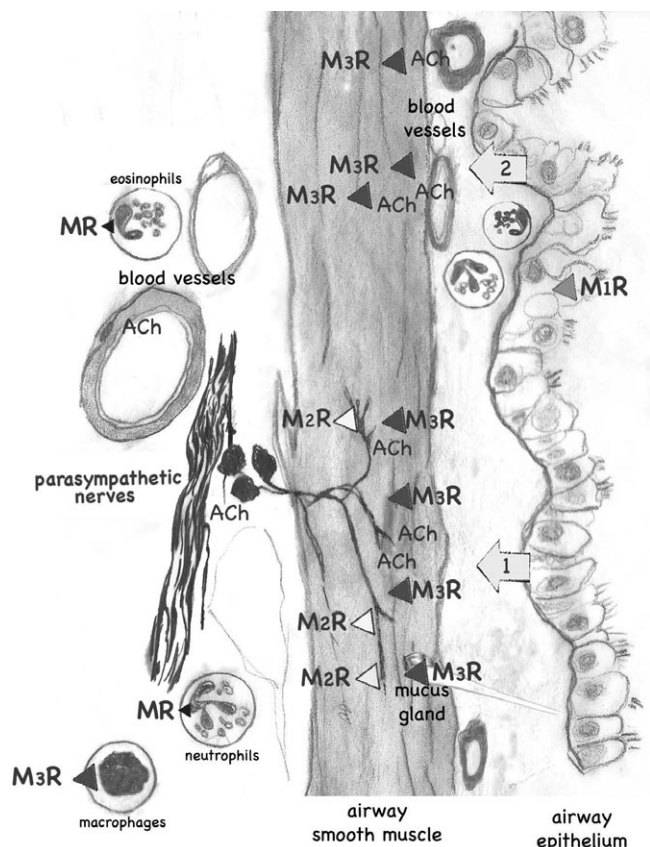
Figure 1

Muscarinic receptors in lungs. Muscarinic receptors (MR) are present throughout the lungs and control smooth muscle contraction, gland secretion, acetylcholine (ACh) release from parasympathetic nerves and probably also inflammatory cells. Only receptors with dominant physiological effects are shown, thus for example M2 receptors in airway smooth muscle are not included. The major physiological source of ACh is from postganglionic parasympathetic nerves that supply both muscle and glands (1); ACh release is normally limited by M2 receptors on these nerves. However, muscarinic receptors are distributed throughout smooth muscle and can be stimulated by exogenous acetylcholine administered i.v. or by inhalation (2). References are found in the text.

et al., 1985; Faulkner *et al.*, 1986). Neuronal M2 receptors are vulnerable, and thus their function is significantly decreased after respiratory viral infection, antigen challenge, or exposure to organophosphates or ozone (Empey *et al.*, 1976; Aquilina *et al.*, 1980; Fryer and Jacoby, 1991; Schultheis, 1992; Schultheis *et al.*, 1994; Sorkness *et al.*, 1994). They are also less functional in humans with asthma (Minette *et al.*, 1989). Decreased function of the neuronal M2 receptors is mediated by various mechanisms including blockade by endogenous antagonists and down-regulation of receptor expression. The resulting increase in acetylcholine release is thought to be an important mechanism of airway hyperreactivity.

Clinically, anticholinergic drugs are used as bronchodilators in combination with anti-inflammatory steroids in the treatment of asthma and chronic obstructive pulmonary disease (COPD). Asthma is characterized by variable airflow limitation that is partially reversible spontaneously or with treatment. Underlying this airflow limitation is chronic inflammation that increases airway hyperresponsiveness to various stimuli (EPR-3, 2007). COPD is characterized by chronic airflow limitation that is not fully reversible. Patients with COPD can experience acute worsening in symptoms. These exacerbations are characterized by increased sputum production and shortness of breath (Rabe *et al.*, 2007). COPD and asthma symptoms overlap; however, the most distinguishing difference between conditions is airflow limitation reversibility. This review covers the history of clinically relevant anticholinergic drugs in asthma and COPD.

Atropine and related compounds

Muscarinic receptor blockade is one of the oldest treatments for asthma. Traditional medicine used the naturally occurring anticholinergic alkaloids atropine and scopolamine for centuries. Ancient Egyptians with airway disease reportedly placed a distillate of henbane, *Hyoscyamus*, on fired bricks and inhaled the smoke (Ebell, 1937; Ellul-Micallef, 1997). In Europe, during the Middle Ages, the source of atropine was the deadly nightshade shrub. This plant was also used as poison, prompting Linnaeus to name it *Atropa belladonna* after Atropos, the Fate that cuts the thread of life (Goodman *et al.*, 2006). In the 19th century, atropine (Figure 2) was isolated from deadly nightshade, datura and jimsonweed in pure form and used in Western medicine.

In both animal and human studies, atropine reverses, in a dose-dependent manner, bronchoconstriction induced by stimulation of parasympathetic nerves or induced by intravenous or inhaled acetylcholine (Cavanaugh and Cooper, 1976; Sheppard *et al.*, 1982; Holtzman *et al.*, 1983), suggesting that anticholinergic drugs would be efficacious in asthma. Here we will discuss why atropine or other anticholinergic medications are not the first line of treatment for airway disease, currently.

The potency of atropine as a bronchodilator depends upon route of administration (Holtzman *et al.*, 1983; Sheppard *et al.*, 1983). It requires more intravenous than inhaled atropine to block bronchoconstriction regardless of whether bronchoconstriction was induced by intravenous acetylcholine or by vagal stimulation (Holtzman *et al.*, 1983). Atropine's potency in asthma is also dependent upon the route of administration. Regardless of whether bronchoconstriction is induced by inhaled methacholine or by vagal reflex (initiated by cold dry air), inhaled atropine is a more effective inhibitor of bronchoconstriction than intravenous atropine (Sheppard *et al.*, 1983). These data might suggest that inhaled atropine would be an ideal bronchodilator. However, and this is an important point of these studies, inhaled atropine is significantly more potent blocking bronchoconstriction induced by inhaled acetylcholine than blocking bronchoconstriction induced by vagal stimulation (Holtzman *et al.*, 1983).

The method used to identify a clinically effective dose of atropine (the dose of any anticholinergic drug that blocks bronchoconstriction induced by inhaled acetylcholine) is significantly less than the dose of atropine required to block the physiological source of acetylcholine, which is the parasympathetic nerves. These experiments in dogs and asthmatic
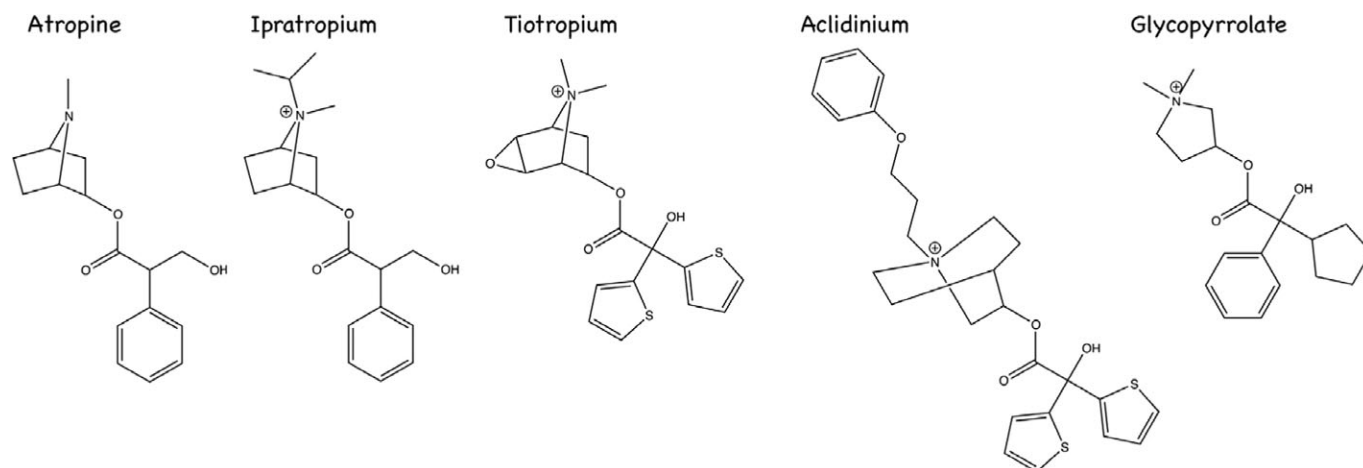
Figure 2

Anticholinergic drugs in asthma and chronic obstructive pulmonary disease.

humans demonstrate the dose of inhaled atropine is too low to block vagally released acetylcholine.

The lack of effectiveness of atropine (and other anticholinergic drugs) in asthma has been used to suggest that increased release of acetylcholine from the parasympathetic does not contribute to hyperreactivity in asthma. However, it is much more likely the clinical dose of atropine is too low. Pharmacologically, 0.67 mg·kg^{-1} (47 mg in a 70 kg adult) of systemic atropine are needed to block 50% of muscarinic receptors in the lungs (Chen et al., 1981). For asystolic arrest the highest clinical dose currently used is 3 mg i.v. per adult (ACC/AHA, 2005). The LD_{50} for atropine is 453 mg per adult; however, 10–20 mg per adult is incapacitating (Goodman, 2010). Thus, the pharmacologically effective dose range for atropine is very close to the toxic range.

Further complicating the dose of atropine is the presence of multiple muscarinic receptor subtypes, including neuronal M2 receptors on parasympathetic nerves supplying the lungs. Under physiological conditions, these neuronal M2 receptors inhibit acetylcholine release, and limit vagally mediated bronchoconstriction (Fryer and Maclagan, 1984; 1987; Minette and Barnes, 1988). In guinea pigs, atropine blocks neuronal M2 receptors and enhances acetylcholine release at doses that have little inhibitory effect on post junctional M3 receptors (Fryer and Maclagan, 1987). Additionally, in humans, atropine has a complex dose–response curve due to the presence of different muscarinic receptor subtypes (Wellstein and Pitschner, 1988).

Atropine is orally absorbed and undergoes first-order kinetics to eliminate the drug (Hinderling et al., 1985a,b). Twenty-four hours after administration, one-fourth to one-third of orally administered atropine is present in urine as a pharmacologically active drug (Kalser, 1971). Systemic side effects of atropine include dry mouth and urinary retention. Atropine also crosses the blood-brain barrier and placenta (Mirakhur, 1978; Proakis and Harris, 1978). It is this ability to penetrate the central nervous system that leads to high fever, hallucinations and coma with higher doses. Although the World Health Organization lists atropine as a core, essential medicine, its therapeutic use is limited to treatment of life-threatening arrhythmias and toxic prodromes due to these multiple, severe, side effects (WHO, 2010). These toxicities severely limit the dose of atropine that can be used clinically. For these reasons there was a push to develop an anticholinergic drug that was less well absorbed to limit toxic side effects.

Ipratropium

Ipratropium bromide (Figure 2) is a synthetic quaternary ammonium compound with an isopropyl group at the N atom of atropine. This quaternary ammonium functions to limit systemic availability to 6.9% when ipratropium is inhaled and limits availability to 2% when taken orally (Ensing et al., 1989). Ipratropium's poor absorption means that it targets muscarinic receptors in the lung when given by inhalation, without the systemic side effects of atropine (Cugell, 1986; Gross, 1988; Goodman et al., 2006). For example, inhaled ipratropium does not affect resting heart rate. The low oral absorption is also important, as approximately 90% of an aerosolized dose is likely swallowed (Davies, 1975; Cugell, 1986). The half-life of ipratropium is 3.2 to 3.8 h regardless of route of administration (Pakes et al., 1980). Onset of action for a >15% increase in forced expiratory volume in 1 s, a measure of airflow limitation, was <15 min, with a peak onset at 1–2 h and duration of action 5 h (Gunther and Kamburoff, 1974; Tashkin et al., 1986).

Ipratropium came into prominent clinic use for COPD in the early 1980s. Part of this use may be because of the relative ineffectiveness of other bronchodilator therapy in COPD. Specifically, β-agonists become less effective with continued use in COPD (Donohue et al., 2003).

However, ipratropium shares the same problem with atropine regarding dose. The FDA limited doses to 18 μg per puff largely due to concerns about potential anticholinergic side effects. Given that the maximal dilating dose of ipratropium is 500 μg, the current recommended dose of 36 μg is suboptimal in COPD patients (Ward et al., 1981; Gross et al., 1989). Side effects for ipratropium are generally mild with dry

mouth and occasional cough most commonly reported. As with β-agonists there is no evidence that regular ipratropium use slows the rate of lung function decline in COPD (Anthonisen *et al.*, 1994).

Ipratropium is less effective in bronchodilation compared to β-agonists (Ruffin *et al.*, 1977). Again, concern for potential side effects limited using a higher dose, thus limiting the bronchodilation. In comparison, acute asthma showed improved outcomes with the addition of ipratropium, to β-agonists, with more rapid and greater improvement in lung function (Rebuck *et al.*, 1987; Stoodley *et al.*, 1999; Rodrigo and Rodrigo, 2000; Rodrigo and Castro-Rodriguez, 2005). However, dosing for acute asthma averaged 504 µg ipratropium per hour and again emphasizes that when ipratropium is used in doses approaching the maximal dilating dose, the bronchodilation is clinically significant (Gross *et al.*, 1989; Rodrigo and Rodrigo, 2000). Current expert opinion now recommends ipratropium for acute severe asthma exacerbations (EPR-3, 2007) at the higher dosing.

However, ipratropium blocks all muscarinic receptor subtypes with equal affinity, including the inhibitory neuronal M2 receptors (Fryer and Maclagan, 1987; Restrepo, 2007). It is via blockade of these neuronal receptors that ipratropium is capable of potentiating vagally induced bronchoconstriction, and this effect is seen at doses that are close to those used clinically (Fryer and Maclagan, 1987). Humans with asthma already have reduced M2 function (Ayala and Ahmed, 1989; Minette *et al.*, 1989), thus to spare additional blockade of neuronal receptors, anticholinergic drugs that are selective for M3 receptors were developed.

Tiotropium

Tiotropium bromide monohydrate is the first anticholinergic drug ever that is effective in treatment of poorly controlled asthma (Peters *et al.*, 2010). This trial showed tiotropium bromide in combination with corticosteroids was more effective than corticosteroids alone and equally effective as corticosteroids in combination with salbutamol, a long-acting beta receptor agonist. Tiotropium is structurally related to ipratropium bromide (Figure 2), but it has a significantly higher affinity for muscarinic receptors (Haddad *et al.*, 1994). Tiotropium has similar affinity for all muscarinic receptor subtypes; however, unlike ipratropium, tiotropium is functionally selective for M3 receptors. This selectivity is provided by the ability of tiotropium to dissociate from M2 receptors 10 times faster than it does from M3 receptors (T1/2 3.6 h for M2 vs. T1/2 34.7 h for M3) (Disse *et al.*, 1993). It has even been suggested that in the lungs, tiotropium is a kinetically irreversible antagonist at M3 muscarinic receptors (Swinney, 2004). This functional muscarinic receptor selectivity is likely related to the two thiophene rings that are a part of tiotropium's structure (Price *et al.*, 2009). Tiotropium has 2–3% bioavailability when taken orally. When inhaled, 80% of tiotropium is swallowed with 19.5% reaching the lung, which is almost entirely bioavailable. Clearance of tiotropium is primarily renal with 14% of an inhaled dose excreted unchanged in the urine with active renal secretion of tiotro-

pium (Price *et al.*, 2009). The advantage of low oral bioavailability and increased renal clearance is fewer systemic side effects.

The prolonged duration of action, higher affinity and functional selectivity of tiotropium for M3 receptors produces greater improvement in airflow limitation when compared to ipratropium (Vincken *et al.*, 2002; Brusasco *et al.*, 2003). Extended half-life of tiotropium allows once daily dosing with subsequent doses progressively increasing efficacy up to 1 week after starting tiotropium (Disse *et al.*, 1993; Haddad *et al.*, 1994; Maesen *et al.*, 1995; Barnes, 2001; Casaburi *et al.*, 2002; Restrepo, 2007). This combination of functional selectivity and extended half-life overcomes many of the drawbacks of ipratropium, including the need for frequent dosing and confounding effects of M2 receptor blockade, and may explain improved outcomes of tiotropium compared with ipratropium in COPD (Casaburi *et al.*, 2002; Vincken *et al.*, 2002; Brusasco *et al.*, 2003).

Asthma is associated with decreased neuronal M2 receptor function leading to increased acetylcholine release (Ayala and Ahmed, 1989; Minette *et al.*, 1989). Tiotropium more rapidly dissociates from M2 receptors than from M3 receptors sparing additional inhibition of neuronal M2 receptors. Thus, unlike other cholinergic antagonists (Fryer and Maclagan, 1987), tiotropium, by not exacerbating acetylcholine release from parasympathetic nerves (Takahashi *et al.*, 1994), further improves bronchodilation.

However, as with all muscarinic antagonists dosing of tiotropium may still be inadequate for treatment of stable asthma. As described above for atropine and ipratropium, dose was determined by the ability of tiotropium to inhibit bronchoconstriction induced by inhaled methacholine and bronchoconstriction induced by i.v. acetylcholine but not vagally induced bronchoconstriction (Barnes *et al.*, 1995; O'Connor *et al.*, 1996; Buels *et al.*, 2010). As with other antimuscarinic drugs 18 µg dose was chosen to limit systemic side effects and not to induce maximal bronchodilation, (Littner *et al.*, 2000) and thus there is potential for under dosing of tiotropium.

In humans tiotropium significantly delayed and reduced COPD exacerbations including hospitalizations for exacerbations (Tashkin *et al.*, 2008). Increased mucus production is a hallmark of COPD exacerbations. Stimulation of muscarinic receptors on epithelial cells promotes cell proliferation, cell survival and mucociliary clearance *in vitro* (Acevedo, 1994; Wessler and Kirkpatrick, 2001; Klein *et al.*, 2009). The role of muscarinic receptors in mucociliary clearance is complex. Mucus glands express M1 and M3 receptors while acetylcholine release from nerves supplying these glands is limited by neuronal M2 receptors. Epithelial cells express M1, M2 and M3 receptors (Acevedo, 1994; Wessler and Kirkpatrick, 2001; Klein *et al.*, 2009). Stimulation of M3 muscarinic receptors increases serous secretions and increases mucociliary beat frequency while M2 receptors inhibit mucociliary beat frequency and decrease particle transport (Klein *et al.*, 2009). The balance of effects of these muscarinic receptors is not fully understood either under physiological or pathological conditions, but does provide opportunity to manipulate secretions with selective muscarinic antagonists. Therefore, as tiotropium has greater affinity for M3 than M1 and M2 receptors this may explain the

reduced exacerbations in COPD (Disse et al., 1999; Tashkin et al., 2008).

Tiotropium was also significantly better than ipratropium in reducing COPD exacerbations when combined with corticosteroids (Tashkin et al., 2008). In antigen challenged animals, tiotropium reduces bronchoconstriction independently of the bronchodilator effects (Buels et al., 2010). This increase effect of tiotropium may result from its anti-inflammatory properties. Muscarinic receptors are found on inflammatory cells in lungs including mast cells (M1), macrophages (M3), neutrophils (M4/M5) and eosinophils (M3/M4) (Mak and Barnes, 1989; Reinheimer et al., 1997; Bany et al., 1999; Verbout et al., 2006). Acetylcholine increases chemotactic mediator leukotriene B4 thereby increasing neutrophil migration. Tiotropium blocks neutrophil migration demonstrating a role for acetylcholine and muscarinic receptors in inflammation (Buhling et al., 2007). Tiotropium reduces airway remodelling that results from prolonged inflammation in allergic guinea pigs (Bos et al., 2007). Severe asthmatic patients responded better to tiotropium than to inhaled corticosteroids further suggesting that tiotropium has anti-inflammatory effects in asthma and COPD (Tashkin et al., 2008; Peters et al., 2010).

Aclidinium bromide

Aclidinium bromide (Figure 2) is an anticholinergic drug similar to tiotropium in that it also has two thiophene rings and quaternary ammonium group (Norman, 2006; Prat et al., 2009). Also similar to tiotropium, aclidinium has kinetic selectivity for M3 receptors versus M2 receptors. Although the half-life of aclidinium at muscarinic receptors in guinea pig lung is 29 h, which is shorter than 34 h for tiotropium, the onset of action is significantly faster (Gavalda et al., 2009). Unlike tiotropium however, aclidinium is rapidly metabolized in the plasma resulting in an extremely short half-life in circulation (2.4 min). This rapid metabolism limits systemic, and central nervous system side effects in animal studies (Gavalda et al., 2009). Early clinical trials appear to confirm a lack of systemic effects (Joos et al., 2010; Schelfhout et al., 2010a), which would allow for higher dosing without the concern for toxic effects that limited earlier use of muscarinic receptor antagonists. Phase I studies in normal patients and in COPD patients showed a 23.3% improvement in airflow limitation 2 h post administration of 300 µg, with sustained bronchodilation over lasting 24 h with once daily dosing (Joos et al., 2010; Schelfhout et al., 2010b). A phase III clinical trial for aclidinium is currently ongoing.

Glycopyrrolate

Glycopyrrolate (Figure 2) has been used in surgery to mitigate the side effects, most notably bradycardia and increased saliva production, of paralytic reversal with neostigmine. Glycopyrrolate is slightly selective for M3 muscarinic receptors with affinity at M3 receptors being 3–5 times higher than that at M1 and M2 receptors (Haddad et al., 1999); however, unlike tiotropium and aclidinium, glycopyrrolate does not

have kinetic selectivity. Glycopyrrolate is currently undergoing phase III trials in COPD (Norman, 2006). A phase II trial shows that 0.5 mg dose of nebulized glycopyrrolate prevented inhaled methacholine-induced bronchospasm 30 h later (Hansel et al., 2005). However, as discussed above with atropine and tiotropium (Holtzman et al., 1983; Sheppard et al., 1983; O'Connor et al., 1996), blocking bronchoconstriction induced by inhaled muscarinic agonists may result in choosing an antagonist dose that is too small to adequately block vagally induced bronchoconstriction (Sheppard et al., 1982; 1983; Holtzman et al., 1983). Accordingly, although glycopyrrolate blocks methacholine-induced bronchoconstriction there is no improvement in bronchoconstriction during acute asthma exacerbations or COPD exacerbations (Cydulka and Emerman, 1994; 1995; Hansel et al., 2005).

Other muscarinic receptor antagonists

Additional long-acting muscarinic receptor antagonists are being developed. These include OrM3 and CHF 5407. OrM3's affinity for M3 receptors is 120 times greater than its affinity for M2 receptors (Table 1). It was formulated in tablets to allow for oral dosing for those patients who had difficulty using inhaled medications. Unfortunately, it was less effective than ipratropium with increased side effects; most notably dry mouth (Lu et al., 2006). CHF 5407 appears more promising. Early trials show it is as potent and long-acting antagonist of M3 receptors as tiotropium (with 54% still bound to M3 receptors at 32 h) with a significantly shorter half-life at M2 receptors (21 min for CHF 5407 vs. 297 min for tiotropium)(Peretto et al., 2007a,b; Cazzola and Matera, 2008). Studies are currently ongoing to determine the clinical effectiveness of CHF 5407.

Formulation of muscarinic receptor antagonists

Inhalation of muscarinic antagonists may not provide optimal delivery of drug to the relevant areas of the lung. Currently anticholinergic drugs are delivered using pressurized metered dose inhaler, dry powder inhalers and a portable nebulizer. These methods result in greater delivery to the lungs versus gastrointestinal tract and different patterns of lung deposition. However, there is no difference in efficacy or side effects of ipratropium or tiotropium with the different inhalers (Vincken et al., 2004; van Noord et al., 2009; Ichinose et al., 2010). Therefore, pharmacology, including receptor selectivity, of muscarinic receptor antagonists is more important than delivery methods to improving clinical efficacy of newer generation muscarinic receptor antagonists.

Conclusion

Rationally, blocking M3 receptors on airway smooth muscle should inhibit bronchoconstriction. However, while anticholinergic drugs are useful in the laboratory setting, their ability

Table 1

Muscarinic receptor (MR) antagonists and properties

	MR affinity at M3 receptor (pK$_i$)	M3 > M2 affinity	Half-life at M3 receptors (h)
Atropine	9.68[1]	None[1]	3.5[7]
Ipratropium	9.58[1]	None[1]	3.2[8]
Tiotropium	11.02[1]	Functional selectivity[4]	34.7[4]
Glycopyrrolate	10.04[1]	3–5× more selective[5]	3.7[5]
Aclidinium	10.74[1]	Functional selectivity[6]	29[6]
OrM3	9.38[2]	120× more selective[2]	14.2[2]
CHF 5407	9.23[3]	Functional selectivity[3]	~32[3]

pK$_i$ determined in heterologous competition experiments against [*N-Methyl*-^{3}H]scopolamine methyl chloride ([^{3}H]NMS).
[1]Casarosa *et al.*, 2009, [2]Lu *et al.*, 2006, [3]Patacchini *et al.*, 2007, [4]Disse *et al.*, 1993, [5]Haddad *et al.*, 1999, [6]Gavalda *et al.*, 2009, [7]Goodman *et al.*, 2006, [8]Pakes *et al.*, 1980.

to block bronchoconstriction clinically in humans with asthma and COPD has been mired with problems surrounding efficacious dosing, side effects and muscarinic receptor selectivity. Each generation of muscarinic antagonists are less well absorbed, more selective, longer acting and, most recently, more readily metabolized. None however, have yet addressed the issue of adequate dosing, and whether antagonists given at concentrations that will inhibit inhaled acetylcholine or methacholine-induced bronchoconstriction will be sufficient to also inhibit bronchoconstriction induced by acetylcholine released from the vagus nerves; the physiological source of acetylcholine in the lungs. Increased acetylcholine is a mechanism of airway hyperreactivity in asthma (Holtzman *et al.*, 1980; Nadel and Barnes, 1984; Minette and Barnes, 1988; Evans *et al.*, 1997; Costello *et al.*, 1999; Yost *et al.*, 1999). Thus, it remains to be seen whether anticholinergic drugs can reach the relevant M3 receptors in lungs *in vivo*, and be delivered in pharmacologically effective concentrations, and produce bronchodilation without toxic side effects.

Conflicts of interest

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