Pulm Ther (2015) 1:103–107
DOI 10.1007/s41030-015-0003-x

BRIEF REPORT

Pharmacological Assessment of the In Vitro Functional Selectivity of Aclidinium Bromide at M_3 and M_2 Muscarinic Receptors in Human Tissue

Amadeu Gavaldà · Elena Gabarda · Javier Milara · Julio Cortijo · Esteban Morcillo · Jorge Beleta · Montserrat Miralpeix

To view enhanced content go to www.pulmonarytherapy-open.com
Received: May 6, 2015 / Published online: September 14, 2015

ABSTRACT

M_3 antagonist activity was assessed in electrically stimulated human bronchial strips; potency, onset and offset of action of aclidinium, tiotropium and ipratropium were determined. M_2 antagonist activity was assessed in electrically stimulated isolated human left atria; duration of action was calculated. Aclidinium demonstrated competitive antagonism at M_3 receptors with similar potency to comparators. Onset of action of aclidinium was similar to ipratropium and faster than tiotropium ($P < 0.05$); duration of action was similar to tiotropium and longer than ipratropium ($P < 0.05$). At M_2 receptors, duration of action of aclidinium was shorter than tiotropium and longer than ipratropium. All antagonists exhibited a shorter duration of action at M_2 versus M_3 receptors. Aclidinium exhibited kinetic selectivity for human bronchial versus atrial receptors, supporting a favorable cardiovascular safety profile.

Keywords: Aclidinium; Atria; Bronchi; Human; In vitro; Muscarinic receptors

Electronic supplementary material The online version of this article (doi:10.1007/s41030-015-0003-x) contains supplementary material, which is available to authorized users.

A. Gavaldà (✉) · J. Beleta · M. Miralpeix
Almirall R&D Centre, Sant Feliu de Llobregat, Barcelona, Spain
e-mail: amadeu.gavalda@almirall.com

E. Gabarda · J. Milara · J. Cortijo · E. Morcillo
Department of Pharmacology, Faculty of Medicine, University of Valencia, Valencia, Spain

J. Milara · J. Cortijo · E. Morcillo
Ciber Respiratory Diseases (CIBERES), Valencia, Spain

J. Milara · J. Cortijo
Research Foundation, University General Hospital, Valencia, Spain

E. Morcillo
Clinical Pharmacology Unit, INCLIVA Research Foundation, University Clinic Hospital, Valencia, Spain

INTRODUCTION

Muscarinic antagonists, such as aclidinium, tiotropium and ipratropium, are known to exert their bronchodilator effects by blocking the actions of acetylcholine at M_3 receptors on airway smooth muscle [1, 2]. However, interactions with muscarinic receptors outside

of the respiratory tract confer a potential for unwanted systemic side effects. For example, blockade of the cardiac M_2 receptors, the principal muscarinic receptor subtype expressed by cardiac myocytes [3–6], induces tachycardia, considered the most severe side effect associated with muscarinic antagonist use [7].

Here, we report the functional potency and duration of action of aclidinium bromide at M_2 and M_3 muscarinic receptors in isolated cardiac and airway tissues of human origin compared with tiotropium and ipratropium.

METHODS

Human lung tissue was harvested from patients undergoing surgery for lung carcinoma and left atrial tissue from patients undergoing cardiac bypass surgery. None of the patients had a history of asthma. The protocol for the use of lung or cardiac tissues was approved by the Ethics Committee of University Clinic Hospital (Valencia, Spain), and informed consent was obtained from all patients.

Fresh bronchial strips were mounted in a superfusion chamber containing oxygenated Kreb's solution (KHS) at 37 °C [8, 9]. Spontaneous tone was inhibited by zileuton 10 μM and fexofenadine 10 μM [10]. Electrical stimulation at 8 Hz, over 0.5 ms and 40–50 V was delivered as 10 s trains of square-wave pulses every 2 min. Once responses to electrical stimulation had stabilized, increasing and cumulative concentrations of aclidinium, ipratropium or tiotropium (0.03–10 nM) were added to the stimulated bronchial strips to measure potency at the M_3 receptors.

To assess onset and offset of action, aclidinium, ipratropium or tiotropium (all 10 nM) were added to the bronchial strips to inhibit approximately 75% of the stable baseline contractions induced by electrical stimulation. After 30 min, the preparation was washed free of antagonist and recovery of tone was recorded for 14–15 h.

Macroscopically normal human left atrial tissue (3–4 mm long) was mounted in a superfusion chamber containing oxygenated KHS at 37 °C (pH 7.4). Each preparation (initial load, 2.0 g) was connected to a force displacement transducer, and changes in tension were recorded. Atrial contraction was induced by electrical stimulation at 1 Hz, over 5 ms and 2–5 V (20% higher than the threshold for contraction). Once responses to electrical stimulation had stabilized (60 min), carbachol 10 μM was added to the stimulated atria to inhibit electrically induced contractions.

To measure the duration of action at the M_2 receptors, aclidinium, ipratropium or tiotropium were added to the carbachol (10 μM)-treated atria at a concentration that inhibited 70% of the maximum carbachol-induced relaxation (70, 80 and 50 nM, respectively). Once inhibition of tone was stable, the antagonists were washed out and the atria incubated with carbachol 10 μM for 240 min.

The rates of onset ($t_{1/2}$) and duration of action (offset) of the antagonists were defined as the time taken from antagonist addition to 50% inhibition of tone, and from antagonist washout to recover 50% ($t_{1/2}$) [8] or maximal recovery (t_{max}) of the maximum carbachol-induced relaxation, respectively. The concentration of antagonist required for 50% inhibition of the electrically stimulated contraction (IC_{50}) and the offset ($t_{1/2}$) was calculated using nonlinear regression analysis.

Statistical significance, set at the 0.05 level, was determined by parametric analysis of

variance, using two-sided statistical tests, followed by Bonferroni's multiple comparison test. All data analyses were performed using GraphPad Prism (San Diego, CA, USA).

RESULTS

Aclidinium, ipratropium and tiotropium produced concentration-dependent relaxation in isolated human bronchial rings. All three muscarinic antagonists inhibited the contractile response induced by electrical stimulation with similar potency, as expressed by IC_{50} values (Table 1).

The duration of action (offset time; at a concentration that inhibited 75% of the electrically stimulated contraction) of aclidinium at the M_3 receptor was significantly longer than that of ipratropium (Table 1; $P < 0.05$), whereas no recovery of tone was observed after washout of tiotropium for the duration of the study. The onset of action of aclidinium was similar to that of ipratropium and significantly faster than tiotropium (Table 1; $P < 0.05$).

The duration of action of aclidinium at the M_2 receptor was significantly longer than that of ipratropium and shorter than that of tiotropium (Table 1; both $P < 0.05$); aclidinium inhibited the bradycardic effect of carbachol with a longer duration of action compared with ipratropium, but shorter than tiotropium (Table 1). Aclidinium had a shorter duration of action at the M_2 receptor than at the M_3 receptor (Table 1).

DISCUSSION

Our results demonstrate that in human bronchial tissue, aclidinium had a similar potency at M_3 receptors to that of tiotropium

Table 1 Duration of action of muscarinic antagonists in isolated human bronchial rings and left atrial strips plus potency in human bronchial rings

Agent	Human bronchial rings					Human left atria strips		
	Strips per patients	IC_{50} (nM)	Maximal inhibition of contraction (%)	Onset time ($t_{1/2}$; min)[a]	Offset time ($t_{1/2}$; min)[b]	Strips per patients	Inhibition of maximum carbachol-induced relaxation (%)	Offset time ($t_{1/2}$; min)[b]
Aclidinium	8/6	0.30 ± 0.11	74.9 ± 3.3	4.4 ± 0.7**	334 ± 49*	3/3	68.4 ± 5.6	110.2 ± 2.5***
Ipratropium	5/3	0.52 ± 0.03	71.1 ± 3.6	3.3 ± 0.6	76 ± 9	3/3	69.8 ± 1.5	16.6 ± 0.3
Tiotropium	5/4	0.26 ± 0.06	76.6 ± 3.9	7.4 ± 1.3*	NR (≥10 h)	3/3	72.1 ± 2.3	159.3 ± 10.5*

Onset and offset in bronchial rings were determined by adding 10 nM of muscarinic antagonist to inhibit approximately 75% of the baseline contraction. Offset in left atrial strips was determined by adding 70, 80 and 50 nM of aclidinium, ipratropium or tiotropium, respectively, to inhibit approximately 70% of the carbachol-induced relaxation. Data reported as mean ± standard error

IC_{50} concentration required for 50% inhibition, *NR* no recovery of tension observed after 10 h

* $P < 0.05$ versus ipratropium

** $P < 0.05$ versus tiotropium

[a] Onset time ($t_{1/2}$) defined as time from antagonist addition to achieve 50% inhibition of tone

[b] Offset time ($t_{1/2}$) defined as the time from antagonist washout to achieve 50% recovery of tone

and ipratropium, although its onset of action was significantly faster than tiotropium and its duration of action was significantly longer than ipratropium. These results are consistent with previous preclinical studies in isolated guinea pig trachea [11, 12]. The faster onset of action of aclidinium compared with tiotropium and the long duration of action of both are consistent with the clinical profile of these compounds [13].

In human left atrial tissue, aclidinium had a shorter duration of action at M_2 receptors than tiotropium, but longer than ipratropium. These results are also consistent with those previously described in isolated guinea pig left atria and in membranes expressing human M_2 receptors [11, 12]. All three antagonists have a faster offset from human left atrial tissue compared with human bronchial tissue, indicating that in human tissue isolates, each compound is kinetically selective. Similar findings have been demonstrated in guinea pig tissue [12], indicating functional selectivity is maintained across species.

CONCLUSION

These results, together with the high plasma hydrolysis rate of aclidinium in comparison with tiotropium [14], may translate into a reduced propensity for systemic effects, particularly unwanted cardiovascular adverse events in the clinical setting, and there is increasing evidence to support this hypothesis [15–17].

ACKNOWLEDGMENTS

This study was funded by Almirall S.A., Barcelona, Spain. The authors would like to thank Caroline D Hewitt, PhD of Complete Medical Communications (Macclesfield, UK), who provided medical writing support under the direction of the authors, funded by Almirall S.A., Barcelona, Spain. EG and JM performed the study and were involved in the acquisition and analysis of the data. AG, JC, EM, JB and MM contributed to study conception, design and data interpretation. AG and MM contributed to the writing of the manuscript. All authors were involved in revising the manuscript for intellectual content. The sponsor was involved in the design of the study, analysis of the data, review of the data and review and approval of the manuscript. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this manuscript, take responsibility for the integrity of the work as a whole and have given final approval for the version to be published.

Disclosures. Amadeu Gavaldà is an employee of Almirall S.A. Jorge Beleta is an employee of Almirall S.A. Montserrat Miralpeix is an employee of Almirall S.A. Elena Gabarda, Javier Milara, Julio Cortijo and Esteban Morcillo have no conflicts of interest to disclose.

Compliance with ethics guidelines. The protocol for the use of lung or cardiac tissues was approved by the Ethics Committee of University Clinic Hospital (Valencia, Spain), and informed consent was obtained from all patients.

REFERENCES

1. Barnes PJ. The role of anticholinergics in chronic obstructive pulmonary disease. Am J Med. 2004;117(Suppl 12A):24S–32S.

2. Roffel AF, Meurs H, Zaagsma J. Identification, localization and function of muscarinic receptor subtypes in airways. In: Zaagsma J, Meurs H, Roffel AF, editors. Muscarinic receptors in airways diseases. Basel, Switzerland: Birkhäuser Verlag; 2001. p. 63–85.

3. Maeda A, Nakai J, Kubo T, et al. Different sensitivities to agonist of muscarinic acetylcholine receptor subtypes. FEBS Lett. 1988;239:399.

4. Entzeroth M, Doods HN, Mayer N. Characterization of porcine coronary muscarinic receptors. Arch Pharmacol. 1990;341:432–8.

5. Gallo MP, Alloatti G, Eva C, Oberto A, Levi RC. M1 muscarinic receptors increase calcium current and phosphoinositide turnover in guinea-pig ventricular cardiocytes. J Physiol. 1993;471:41–60.

6. Yang CM, Chen FF, Sung TC, Hsu HF, Wu D. Pharmacological characterization of muscarinic receptors in neonatal rat cardiomyocytes. Am J Physiol. 1993;265:C666–73.

7. Eglen RM. Muscarinic receptor subtype pharmacology and physiology. Prog Med Chem. 2005;43:105–36.

8. Coleman RA, Nials AT. Novel and versatile superfusion system. Its use in the evaluation of some spasmogenic and spasmolytic agents using guinea-pig isolated tracheal smooth muscle. J Pharmacol Method. 1989;21:71–86.

9. Coleman RA, Nials AT, Rabe KF, Vardey CJ, Watson N. Isolated, electrically-stimulated airway preparations—their use in determining beta-adrenoceptor agonist activity. Pulm Pharmacol. 1996;9:107–17.

10. Ellis JL, Undem BJ. Role of cysteinyl-leukotrienes and histamine in mediating intrinsic tone in isolated human bronchi. Am J Respir Crit Care Med. 1994;149:118–22.

11. Gavaldà A, Miralpeix M, Ramos I, et al. Characterization of aclidinium bromide, a novel inhaled muscarinic antagonist, with long duration of action and a favorable pharmacological profile. J Pharmacol Exp Ther. 2009;331:740–51.

12. Gavaldà A, Ramos I, Carcasona C, et al. The in vitro and in vivo profile of aclidinium bromide in comparison with glycopyrronium bromide. Pulm Pharmacol Ther. 2014;28:114–21.

13. Fuhr R, Magnussen H, Sarem K, et al. Efficacy of aclidinium bromide 400 mg twice daily compared with placebo and tiotropium in patients with moderate to severe COPD. Chest. 2012;141:745–52.

14. Sentellas S, Ramos I, Albertí J, et al. Aclidinium bromide, a new, long-acting, inhaled muscarinic antagonist: in vitro plasma inactivation and pharmacological activity of its main metabolites. Eur J Pharm Sci. 2010;39:283–90.

15. Lasseter KC, Aubets J, Chuecos F. Garcia Gil E. Aclidinium bromide, a long-acting antimuscarinic, does not affect QT interval in healthy subjects. J Clin Pharmacol. 2011;51:923–32.

16. Kerwin EM, D'Urzo AD, Gelb AF, et al. Efficacy and safety of a 12-week treatment with twice-daily aclidinium bromide in COPD patients (ACCORD COPD I). COPD. 2012;9:90–101.

17. Jones PW, Singh D, Bateman ED, et al. Efficacy and safety of twice-daily aclidinium bromide in COPD patients: the ATTAIN study. Eur Respir J. 2012;40:830–6.