

# Methacholine challenge testing: comparative pharmacology

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**Abstract:** Standardization of the methacholine inhalation challenge, the most common direct bronchoprovocation test, is important. One aspect of standardization is the appropriate washout period for pharmacologic agents which affect the response. This review summarizes the available data on pharmacologic inhibition of the methacholine response. Specific (anti-muscarinic) agents demonstrate marked bronchoprotection (up to 7 days for the long-acting drugs) which lasts longer than the duration of bronchodilation. The functional antagonist (beta 2 agonist class of medications) shows marked, but less, bronchoprotection which is relatively short lived and is similar to the duration of bronchodilator efficacy. Tolerance develops quickly, especially to the long-acting agents. Single doses of controller medications, such as inhaled corticosteroids (ICS) and leukotriene receptor antagonists, have no effect on the methacholine test, while regular use, at least for ICS, has a modest protective effect whose duration is uncertain and likely variable. Theophylline has a small effect and H1 blockers (all generations) have a negligible effect. **Keywords:** methacholine challenge, bronchoprotection, muscarinic antagonist, beta agonist, glucocorticosteroid, antihistamine

## Introduction

The methacholine inhalation challenge test is widely used both in clinical and in research settings to measure direct airway responsiveness.<sup>1</sup> The results are traditionally expressed as the provocation dose (PD) or concentration (PC) that results in a 20% fall in the forced expiratory volume in 1 second (FEV<sub>1</sub>), the PD<sub>20</sub> or PC<sub>20</sub>. The PD<sub>20</sub> has short-term repeatability of  $\pm 1$ –1.5 doubling doses, mostly due to lack of precision rather than genuine variation. Adequate standardization of the test is therefore important to assure the best discrimination between normal and increased responsiveness and to compare results between different methods. Standardization documents have been produced by the American Thoracic Society<sup>2</sup> and more recently updated by the European Respiratory Society.<sup>3</sup> One important aspect of standardization is the withhold time for various respiratory and non-respiratory medications which may affect the test. We found that in preparing both the 2000 and 2017 documents, data regarding this were frequently lacking or at best incomplete. This prompted several of our own investigations as well as this review article.

Airway hyper-responsiveness (AHR) to methacholine is defined as an increase in sensitivity (left shift of the dose–response curve, ie, PD<sub>20</sub>/PC<sub>20</sub>), reactivity (slope of the curve), and/or increase and eventual loss of the maximal dose–response plateau.<sup>4</sup> AHR is a characteristic feature of asthma. Clinically, the methacholine challenge test (MCT) is highly sensitive with a high negative predictive value and is particularly useful

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to exclude a diagnosis of “current” asthma when the test is negative.<sup>1-3</sup> In research, the MCT is used to identify eligible study participants, assess changes in AHR following allergen exposure, or determine the bronchoprotective effect of novel compounds. Methacholine challenge testing has also been used to investigate therapeutic bioequivalence<sup>5</sup> and may have a role in the evaluation and management of severe asthma.<sup>6</sup> Pharmacological agents will inhibit or suppress the response to methacholine by specific antagonism (eg, anti-muscarinic agents), by functional antagonism (eg, other bronchodilators, especially beta agonists), or by an anti-inflammatory effect (eg, corticosteroids). Potentially, any/all aspects of the methacholine response may be affected; however, the large majority of studies address the PD<sub>20</sub>/PC<sub>20</sub> (sensitivity). The purpose of this communication is to provide a reference for the comparative pharmacology of various respiratory medications on (primarily clinical) methacholine challenge testing.

## Bronchodilators

### Muscarinic antagonists – short acting

Inhaled methacholine induces bronchoconstriction in a manner analogous to that of acetylcholine. Methacholine binds airway smooth muscle (ASM) muscarinic receptors, importantly the M<sub>3</sub> subtype, triggering a cascade of intracellular signals that ultimately leads to the release of calcium and ASM contraction. The result is a decrease in airway diameter and an increase in resistance to airflow that can be quantitated by simple spirometry. Anticholinergic agents

or muscarinic antagonists inhibit this response. The use of atropine-containing cigarettes for treating bronchospasm was an early indication of anticholinergic efficacy.<sup>7</sup> Other early investigations using more controlled methodology, although not as refined as that used today, also showed the effectiveness of atropine.<sup>8-10</sup> Ipratropium bromide (IB; formerly SCH1000), developed in the early 1970s, was the first modern inhaled muscarinic receptor antagonist for relieving bronchoconstriction. Each actuation of the pressurized metered dose inhaler (pMDI) device delivers a 20 µg dose. The standard dose is 40 µg as needed. The bronchoprotective effects of IB against inhaled methacholine have varied with respect to mode of administration, dose, time point of measurement, and end point (Table 1). Following a standard dose of IB via pMDI, an average of 2.5 doubling concentration protection from methacholine-induced bronchoconstriction (MIB) has been shown at 20 and 60 minutes post-dose.<sup>11,12</sup> An earlier study in nine asthmatic children using twice the standard dose (80 µg) of SCH1000 via pMDI showed complete inhibition of the response (i.e, flat dose–response curve) at 30 minutes post-dose. The limitations on quantifying the response were twofold: first, the maximum concentration of methacholine used was 25 mg/mL and second, participants had, on average, relatively mild baseline airway responsiveness (mean methacholine PD<sub>20</sub> [provocative dose causing a 20% fall in forced expiratory volume] of 13.9 µg) at baseline.<sup>13</sup> A subsequent study used concentrations of methacholine up to 362 mg/mL. In this adult population, the mean shift in methacholine PC<sub>20</sub>

**Table 1** Bronchodilator: SAMAs

Agent	Device	Dose (µg)	End point	Time point (hours)	Dose shift (doubling concentrations)	Reference (year)
Ipratropium	pMDI	80	PD <sub>20</sub>	0.5	NQ	Woenne et al <sup>13</sup> (1978)
Ipratropium	pMDI	80	PC <sub>20</sub>	1	5.8	Bandouvakis et al <sup>14</sup> (1981)
Ipratropium	pMDI and DPI	80	sGaw	0.75	~4	Larsson <sup>15</sup> (1987)
Ipratropium	pMDI	200	sGaw	0.75	4	Larsson <sup>15</sup> (1987)
Ipratropium	DPI	200	sGaw	0.75	5	Larsson <sup>15</sup> (1987)
Ipratropium	pMDI	40	PD <sub>20</sub>	0.33	2.3	Crimi et al <sup>11</sup> (1992)
Ipratropium	DeVilbiss 700 Nebulizer	500	PC <sub>20</sub>	2	5	Hansel et al <sup>16</sup> (2005)
				12	0	
				24	0	
				30	0	
Ipratropium	pMDI	40	PD <sub>15</sub>	1	2.7	Sposato et al <sup>12</sup> (2008)
Ipratropium	Nasal spray	0.03% (2/nare)	PC <sub>20</sub>	0.16	<0.5	Reid et al <sup>19</sup> (2005)
Ipratropium	pMDI	40	PC <sub>20</sub>	6	1	Ilamperuma et al <sup>18</sup> (2009)
				12	<0.5	
Oxipropium	pMDI	200	PD <sub>15</sub>	1	4.2	Sposato et al <sup>12</sup> (2008)

**Abbreviations:** SAMAs, short-acting muscarinic antagonists; pMDI, pressurized metered dose inhaler; DPI, dry powder inhaler; PD<sub>20</sub>, provocation dose that results in a 20% fall in the forced expiratory volume in 1 second; PC<sub>20</sub>, provocation concentration that results in a 20% fall in the forced expiratory volume in 1 second; sGaw, airway conductance; PD<sub>15</sub>, dose of methacholine producing a 15% decrease in forced expiratory volume in 1 second; NQ, not quantifiable.

(provocative concentration causing a 20% fall in forced expiratory volume) was 5.8 doubling concentrations at 60 minutes following pMDI inhalation of 80 µg of IB.<sup>14</sup> Larsson et al investigated bronchoprotection 45 minutes after 80 or 200 µg IB administered via pMDI or dry powder inhaler (DPI).<sup>15</sup> Using a 35% reduction in specific airway conductance (sGaw PC<sub>35</sub>) as the end point, they showed similar effects on airway sensitivity to methacholine (4–5 doubling concentrations) that were independent of dose and device. The protection afforded by 500 µg of nebulized IB and assessed at 2 hours post-dose<sup>16</sup> was similar to that shown by Larsson. Although the exact magnitude of bronchoprotection following a 500 µg dose of nebulized atropine could not be quantified (low threshold concentration of 16 mg/mL), a minimum shift of 5–6 doubling concentrations has been observed; this shift is about 3–4 doubling concentrations greater than that following the same dose administered intravenously.<sup>17</sup> On average, the protective effect is diminished to one doubling concentration at 6 hours following a standard dose.<sup>18</sup> Half of those studied at 6 hours showed greater than the average protection and none showed significant protection at 12 hours post-dose. Bronchoprotection following nebulized high dose is also negligible by 12 hours.<sup>16</sup> Nasal administration of a 0.3% solution showed a slight statistically significant but clinically irrelevant inhibitory effect on the MCT 10 minutes after dosing.<sup>19</sup> Another short-acting muscarinic antagonist (SAMA) is oxitropium bromide (OB). At five times the standard dose of IB, OB produces roughly 1.5 times the bronchoprotection against MIB 1 hour after dosing (OB 4.2 vs IB 2.7 doubling concentrations).<sup>12</sup> The SAMA data are summarized in Table 1.

## Muscarinic antagonists – long acting

Long-acting muscarinic antagonists (LAMAs) include tiotropium (TIO), glycopyrronium, aclidinium, and umeclidinium. Classification based on duration of action relates to the bronchodilator property of these agents. This is reflected in the dosing regimen and determined through the clinical development process. TIO, for example, is dosed once per day (ie, bronchodilator effects last >24 hours). On the other hand, bronchodilation following IB is on the order of 6 hours and dosing can be 3–4 times per day. As indicated earlier, protection against MIB following a standard dose of IB is reduced at 6 hours and therefore consistent with the duration of bronchodilation. This does not appear to be the case with LAMAs where bronchodilation is short-lived relative to the bronchoprotective property. O'Connor et al studied three doses of TIO (10, 40, and 80 µg) vs placebo in random order with each delivered as a single dose via the

DPI 2 hours prior to methacholine challenge testing.<sup>20</sup> Non-linear dose-dependent bronchoprotection was evident across the dosing range (5.0, 7.1, and 7.9 doubling concentration shifts for 10, 40, and 80 µg respectively). The protection, although decreased to 2.2, 2.2, and 3.0 doubling concentrations, persisted to 48 hours and in a small subpopulation of the study cohort (n=4) was still evident at 72 hours. A mild dose-independent bronchodilator effect was noted but the effect did not surpass 24 hours. TIO delivered via the DPI showed slightly less maximal bronchoprotection (4.1 doubling dose shift) following a single 18 µg dose.<sup>12</sup> This effect has also been observed with a single 5 µg dose of TIO delivered via the Respimat® inhaler (Boehringer Ingelheim, Ingelheim am Rhein, Germany; 4.2 doubling concentration shift).<sup>21</sup> The Blais et al study followed the duration of bronchoprotection to 168 hours (ie, 7 days) and found the effect to be small but still present and statistically significant. This was a comparative study with glycopyrronium (single dose of 50 µg via DPI). Glycopyrronium produced similar maximal bronchoprotection to MIB at 1 hour (4.3 doubling concentrations) and significant protection, although comparatively less, at subsequent time points (24, 48, 72, and 96 hours). In contrast to the bronchoprotective effect of TIO at 168 hours, the small protective effect of glycopyrronium observed at 168 hours was not significant. Glycopyrronium bronchoprotection at 1, 24, and 48 hours was reproduced by this same group in a subsequent study.<sup>22</sup> Neither treatment produced clinically significant bronchodilation, possibly explained by the mild asthma study population.

The bronchoprotective properties of high-dose nebulized racemic glycopyrrolate (500, 1000, 2000 µg) have also been studied.<sup>16</sup> Maximal protection against MIB was similar to that shown by standard doses administered with the DPI or Respimat. The highest dose produced the least bronchodilation (~5% at 1–2 hours). The two lower doses improved the 1–2 hour post-dose FEV<sub>1</sub> by about 10%.

As would be expected, inhaled selective muscarinic antagonists like IB and TIO decrease airway sensitivity to methacholine and, on average, produce a maximal shift in the dose–response curve, about 5 doubling concentrations (ie, about 32-fold) to the right. The maximal effect appears to be independent of both the dose and the delivery device. Although not widely studied, protection against MIB bronchoconstriction following single-dose SAMA is minimal at 12 hours and that following single-dose LAMA lasts 7 days. This difference is presumably explained by the prolonged binding and slow dissociation of the LAMA from the muscarinic receptor. Receptor downregulation may also

be a consequence of prolonged LAMA/M<sub>3</sub> binding and may contribute to a decrease in the response to inhaled methacholine. The LAMA data are summarized in Table 2.

There are limited data on other aspects of the methacholine dose–response curve. Both TIO and glycopyrronium resulted in the appearance of a plateau response 1 hour after administration.<sup>21</sup> The mechanism of this finding is uncertain.

## Beta agonists – short acting

Beta agonists prevent ASM contraction through “functional” antagonism. These agents are sympathomimetic and bind ASM adrenergic beta 2 receptors. The beta 2 receptor is a G protein (G $\alpha_s$ ) coupled receptor that activates adenylyl cyclase,

leading to an increase in the level of intracellular cyclic adenosine monophosphate (cAMP). Cyclic AMP in turn activates protein kinase A, which prevents/reverses ASM contraction by at least two mechanisms: the first is activation of a transmembrane calcium/potassium exchange/channel that decreases the amount of intracellular calcium and the second is inhibition of myosin light chain kinase. Beta agonists used in respiratory illness have also evolved to include both short- and long-acting formulations. Salbutamol (pMDI) and terbutaline (DPI) are commonly used short-acting beta agonists (SABAs). A standard dose of 200  $\mu$ g salbutamol delivered via pMDI shifts the methacholine dose–response curve, on average, about 3.5 doubling concentrations to the right when

**Table 2** Bronchodilator: long-acting muscarinic antagonists (including combination LAMA/LABA)

Agent	Device	Dose ( $\mu$ g)	End point	Time point (hours)	Dose shift (doubling concentrations)	Reference (year)
Tiotropium	DPI	10	PC <sub>20</sub>	2	5.0	O'Connor et al <sup>20</sup> (1996)
		40			7.1	
		80			7.9	
Tiotropium	DPI	18	PD <sub>15</sub>	1	4.1	Sposato et al <sup>12</sup> (2008)
Tiotropium	Respimat	5	PC <sub>20</sub>	1	4.2	Blais et al <sup>21</sup> (2016)
				24	3.1	
				48	2.7	
				72	2.3	
				96	1.9	
				168	0.84	
Glycopyrronium	DPI	50	PC <sub>20</sub>	1	4.3	Blais et al <sup>21</sup> (2016)
				24	1.8	
				48	1.9	
				72	1.2	
				96	1.1	
				168	0.52	
Glycopyrronium	DPI	50	PC <sub>20</sub>	1	5	Blais et al <sup>22</sup> (2017)
				24	2	
				48	2	
Racemic glycopyrrolate	DeVilbiss 700 Nebulizer	500	PC <sub>20</sub>	2	4	Hansel et al <sup>16</sup> (2005)
				12	2	
				24	1	
				30	1	
Racemic glycopyrrolate	DeVilbiss 700 Nebulizer	1000	PC <sub>20</sub>	2	6	Hansel et al <sup>16</sup> (2005)
				12	4	
				24	3.5	
				30	3	
Racemic glycopyrrolate	DeVilbiss 700 Nebulizer	2000	PC <sub>20</sub>	2	5	Hansel et al <sup>16</sup> (2005)
				12	3	
				24	3	
				30	2	
LAMA/LABA combination	DPI	50	PC <sub>20</sub>	1	5	Blais et al <sup>22</sup> (2017)
	DPI	75		24	2	
	Glycopyrronium Indacaterol			48	2	

**Abbreviations:** LAMA, long-acting muscarinic antagonists; LABA, long-acting beta antagonists; DPI, dry powder inhaler; PC<sub>20</sub>, provocation concentration that results in a 20% fall in the forced expiratory volume in 1 second; PD<sub>15</sub>, provocation dose that results in a 15% fall in the forced expiratory volume in 1 second.

assessed at 10<sup>23–25</sup> or 30 minutes post-dose (range 2.4–4.6).<sup>26</sup> The effect diminishes to 1.9 doubling concentrations at 1 hour<sup>27</sup> and is absent at 12 hours.<sup>26</sup> Smaller (100 µg) and larger (400 µg) doses produce similar inhibition (2.9–4.5 doubling concentrations, respectively).<sup>23</sup> Large nebulized doses of R (1.25 mg) and racemic salbutamol (2.5 mg) provide the same protection at 20 minutes as a standard dose delivered via pMDI (3.3 and 3.4 doubling concentrations, respectively).<sup>28</sup> A high nebulized dose of the S isomer, although showing some effect (0.9 doubling concentrations), contributes little to the overall bronchoprotective effect afforded by the racemic molecule.<sup>28</sup> When reassessed at 3 hours, the S isomer was ineffective and the protection produced by both racemic salbutamol and R salbutamol had decreased to about 1 doubling concentration. A subsequent study using standard doses of racemic (200 µg), R (100 µg), and S (100 µg) delivered via nebulizer confirmed the ineffectiveness of the S isomer and showed shifts in methacholine PC<sub>20</sub> of ~3 doubling concentrations for both the R isomer and the racemic molecule.<sup>29</sup> At 1.5 hours post-dose, bronchoprotection against MIB provided by a standard dose of terbutaline (500 µg) via pMDI is similar to that shown with 200 µg salbutamol at 1 hour post-dose (ie, ~1.8 doubling concentration shift).<sup>30,31</sup> This is about 1 doubling concentration less than that shown at 20 minutes following a single dose of 500 µg.<sup>32</sup> The bronchoprotection afforded by terbutaline is essentially gone by 6 hours.<sup>30</sup> Comparison of maximal bronchoprotection between terbutaline and salbutamol is not possible from currently available data as the effect with terbutaline was not studied at earlier time points. One would anticipate that similar bronchoprotection would be observed. A large dose of fenoterol (800 µg via pMDI, twice the standard dose) produced a 4 doubling concentration shift in methacholine PC<sub>20</sub> at 1 hour post-dose.<sup>14</sup> The SABA data are summarized in Table 3.

## Beta agonists – long and ultra long acting

Long-acting beta agonists (LABAs) include salmeterol and formoterol. The more recently developed ultra-long-acting formulations or uLABAs include indacaterol, olodaterol, and vilanterol. Salmeterol appears to provide a similar magnitude of bronchoprotection as its short-acting counterpart salbutamol but at a much smaller dose and for a longer duration. For example, 50 µg salmeterol via pMDI produces a 4 doubling concentration shift in methacholine PC<sub>20</sub> at 30 minutes, and this protection is maintained for at least 12 hours.<sup>26,33</sup> Others have shown a 3.3 doubling concentration shift at 1 hour after 50 µg salmeterol.<sup>34,35</sup> Halving the dose had no effect on the duration of protection but decreased the magnitude of protection by about twofold, from 4 doubling

concentrations to 3 doubling concentrations.<sup>26</sup> Others have shown less bronchoprotection at equal (50 µg) and higher (100 µg) doses.<sup>27</sup> A 24 µg dose of formoterol, a dry powder formulation, provides comparable bronchoprotection to that seen with salmeterol (ie, ~4 doubling doses at 1 hour post-dose).<sup>5,31,36</sup> MIB is inhibited with lower doses (6 and 12 µg), about twofold less than the 24 µg dose, and there is little difference in the magnitude of bronchoprotection between the two lower doses; both provide about 2.5 doubling concentration shift.<sup>5,36,37</sup> With respect to the duration of action of formoterol, a 12 µg dose still shows about 1 doubling concentration protection at 8 hours post-dose. There are limited data for the uLABAs. Olodaterol (BI1744) at single doses of 2, 5, 10, and 20 µg, delivered via a soft mist inhaler, provided dose-dependent inhibition of MIB for at least 32 hours in the range of 2.0 doubling concentrations for the low dose up to 4.2 doubling concentrations for the high dose.<sup>38</sup> Maximal protection with each dose was evident 30 minutes post-inhalation. A single dose of 75 µg indacaterol was much less impressive. The shift in methacholine PC<sub>20</sub> was 1.5 doubling concentrations at 1 hour post-dose and this decreased to 1 doubling concentration on subsequent testing at both 24 and 48 hours.<sup>22</sup>

From these data, a single dose of 200µg salbutamol provides rapid and significant bronchoprotection against MIB that resolves well before 12 hours. As was shown with terbutaline, the effect is most likely gone by 6 hours. The long-acting agent salmeterol shows similar efficacy for inhibiting MIB and the effect is unchanged at 12 hours. At standard doses, the uLABAs olodaterol (5 µg) and indacaterol (75 µg) provide less protection than LABAs or SABAs. However, high doses of olodaterol provide equipotent bronchoprotection to that seen with salmeterol and salbutamol. The duration of efficacy with uLABAs is on the order of 32–48 hours and this may apply to the LABA agents as well. In contrast to the LAMA mechanism, the LABA/uLABA effect is not a direct result of receptor binding characteristics, as muscarinic receptors are unopposed in the presence of beta agonist; however, the effect may be explained by a prolonged increase in cAMP or decreases in intracellular signaling molecules required for ASM contraction (eg, calcium). An important and well-documented phenomenon associated with beta agonists is the loss of bronchoprotection following regular use.<sup>32,34,35</sup> Tolerance develops quickly, is greater with LABAs<sup>34,35</sup> than with SABAs<sup>20,24</sup> and should be given consideration when interpreting responses to methacholine challenge testing. The LABA data are summarized in Table 4.

Several studies have addressed the effect of beta 2 agonists on other aspects of the methacholine dose–response

**Table 3** Bronchodilator: SABAs

Agent	Device	Dose (µg)	End point	Time point (hours)	Dose shift (doubling concentrations)	Reference (year)
Salbutamol	MDI	200	PC <sub>20</sub>	1	1.9	Derom et al <sup>27</sup> (1992)
Salbutamol	pMDI	200	PC <sub>20</sub>	0.5	3.5	Simons et al <sup>26</sup> (1992)
				12	0	
Salbutamol	pMDI	200	PC <sub>20</sub>	0.16	4.6	Parameswaran et al <sup>23</sup> (1999)
Salbutamol	pMDI	100	PC <sub>20</sub>	0.16	2.9	Parameswaran et al <sup>23</sup> (1999)
		200			3.9	
		400			4.1	
Salbutamol	pMDI-HFA	100	PC <sub>20</sub>	0.16	3.1	Parameswaran et al <sup>23</sup> (1999)
		200			3.9	
		400			4.5	
Salbutamol	pMDI	200	PC <sub>20</sub>	0.16	3.5	Jokic et al <sup>24</sup> (2001)
Salbutamol	pMDI	200	PC <sub>20</sub>	0.16	2.4	Stewart et al <sup>25</sup> (2012)
Salbutamol	pMDI	200	PC <sub>20</sub>	1	1.9	Derom et al <sup>27</sup> (1992)
Salbutamol	Nebulized	2500	PC <sub>20</sub>	0.33	~3.4	Cockcroft <sup>28</sup> (1997)
R-salbutamol	Nebulized	1250	PC <sub>20</sub>	0.33	~3.3	Cockcroft and Swystun <sup>28</sup> (1997)
S-salbutamol	Nebulized	1250	PC <sub>20</sub>	0.33	0.9	Cockcroft <sup>28</sup> (1997)
Salbutamol	Nebulized	2500	PC <sub>20</sub>	3	1.0	Cockcroft and Swystun <sup>28</sup> (1997)
R-salbutamol	Nebulized	1250	PC <sub>20</sub>	3	1.2	Cockcroft and Swystun <sup>28</sup> (1997)
S-salbutamol	Nebulized	1250	PC <sub>20</sub>	3	0	Cockcroft and Swystun <sup>28</sup> (1997)
Salbutamol	Nebulized	200	PC <sub>20</sub>	0.5	2.8	Ramsay <sup>29</sup> (1999)
R-salbutamol	Nebulized	100	PC <sub>20</sub>	0.5	2.9	Ramsay et al <sup>29</sup> (1999)
S-salbutamol	Nebulized	100	PC <sub>20</sub>	0.5	0.15	Ramsay et al <sup>29</sup> (1999)
Terbutaline	DPI	500	PD <sub>20</sub>	1	1.8	Lipworth et al <sup>36</sup> (1998)
Terbutaline	Turbuhaler	250	PC <sub>20</sub>	1.5	1.45	Derom et al <sup>30</sup> (2001)
				3	0.5	
				6	-0.5	
Terbutaline	Turbuhaler	500	PC <sub>20</sub>	1.5	1.67	Derom et al <sup>30</sup> (2001)
				3	0.75	
				6	0	
Terbutaline	pMDI	250	PC <sub>20</sub>	1.5	0.99	Derom et al <sup>30</sup> (2001)
				3	0.13	
				6	-0.8	
Terbutaline	pMDI	500	PC <sub>20</sub>	1.5	1.54	Derom et al <sup>30</sup> (2001)
				3	0.25	
				6	0	
Terbutaline	Turbuhaler	500	PC <sub>20</sub>	0.33	2.7	O'Connor et al <sup>32</sup> (1992)
Fenoterol	pMDI	800	PC <sub>20</sub>	1	4	Bandouvakis et al <sup>14</sup> (1981)

**Abbreviations:** SABAs, short-acting beta agonists; MDI, metered dose inhaler; pMDI, pressurized metered dose inhaler; HFA, hydrofluoroalkane; DPI, dry powder inhaler; PD<sub>20</sub>, provocation dose that results in a 20% fall in the forced expiratory volume in 1 second; PC<sub>20</sub>, provocation concentration that results in a 20% fall in the forced expiratory volume in 1 second.

curve.<sup>22,39,40</sup> All three studies confirm that beta agonists do not lead to a methacholine dose–response plateau, and that they may actually increase the steepness of the dose–response curve (ie, reactivity).<sup>39</sup>

## Xanthines

The use of theophylline as a bronchodilator has largely been replaced by inhaled agents. Nonetheless, as a phosphodiesterase enzyme inhibitor, there is a mechanistic interest surrounding the effect of theophylline on methacholine challenge testing. Oral theophylline administered over 2 months at a median dose of 250 mg/day increases methacholine PC<sub>20</sub>

by 3.2 fold.<sup>41</sup> Acute effects following intravenous administration of 5, 10, and 15 mg/L are probably minimal<sup>42</sup> (Table 5).

## Anti-inflammatory and other controller treatments

### Corticosteroids

Inhaled corticosteroids (ICS) are the gold standard for decreasing airway inflammation. ICS that are currently in use and for which an effect on MIB has been studied include budesonide, fluticasone, beclomethasone, and ciclesonide. In children, a single 800 µg dose of budesonide via pMDI with spacer had no effect on methacholine PD<sub>20</sub> 2 hours

**Table 4** Bronchodilator: long-acting beta agonists (LABAs)

Agent	Device	Dose (µg)	End point	Time point (hours)	Dose shift (doubling concentrations)	Reference (year)
Salmeterol	pMDI	25	PC <sub>20</sub>	0.5	~3	Simons et al <sup>26</sup> (1992)
				12	~3	
Salmeterol	pMDI	50	PC <sub>20</sub>	0.5	~4	Simons et al <sup>26</sup> (1992)
				12	~4	
Salmeterol	pMDI	50	PC <sub>20</sub>	1	1.9	Derom et al <sup>27</sup> (1992)
				100	2.7	
Salmeterol	pMDI	50	PD <sub>20</sub>	1	~4	Verbene et al <sup>33</sup> (1993)
Salmeterol	pMDI (with chamber)	50	PC <sub>20</sub>	1	3.3	Cheung et al <sup>34</sup> (1992)
Salmeterol	pMDI	50	PC <sub>20</sub>	1	3.3	Bhagat et al <sup>35</sup> (1992)
Formoterol	DPI	6	PD <sub>20</sub>	1	2.5	Lipworth et al <sup>36</sup> (1998)
				12	2.7	
				24	3.3	
Formoterol	DPI	12	PC <sub>20</sub>	0.16	~2	Davis et al <sup>37</sup> (2003)
Formoterol	pMDI	12	PC <sub>20</sub>	0.5	3.8	Lipworth et al <sup>31</sup> (2005)
				8	1.2	
				1	2.6	
Formoterol	DPI	24	PC <sub>20</sub>	1	3.8	Prabhakaran et al <sup>5</sup> (2011)
Indacaterol	DPI	75	PC <sub>20</sub>	1	1.5	Blais et al <sup>22</sup> (2017)
				24	1	
				48	1	
Olodaterol	Respimat	2	PC <sub>20</sub>	0.5	2.1	O'Byrne et al <sup>38</sup> (2009)
				4	2.0	
				8	1.9	
				24	1.2	
				32	1.2	
Olodaterol	Respimat	5	PC <sub>20</sub>	0.5	2.6	O'Byrne et al <sup>38</sup> (2009)
				4	2.4	
				8	2.5	
				24	1.7	
				32	1.8	
Olodaterol	Respimat	10	PC <sub>20</sub>	0.5	3.6	O'Byrne et al <sup>38</sup> (2009)
				4	3.6	
				8	3.3	
				24	2.5	
				32	2.1	
Olodaterol	Respimat	20	PC <sub>20</sub>	0.5	4.2	O'Byrne et al <sup>38</sup> (2009)
				4	4.2	
				8	4.2	
				24	3.0	
				32	2.7	

**Abbreviations:** pMDI, pressurized metered dose inhaler; DPI, dry powder inhaler; PD<sub>20</sub>, provocation dose that results in a 20% fall in the forced expiratory volume in 1 second; PC<sub>20</sub>, provocation concentration that results in a 20% fall in the forced expiratory volume in 1 second.

post-dose and produced a negligible shift in PD<sub>20</sub> (0.1 doubling doses) at 5 hours.<sup>43</sup> A shift of 0.79 doubling concentrations in methacholine PC<sub>20</sub> occurred following 8 weeks of low-dose budesonide (200 µg/day);<sup>44</sup> administering twice the dose for half the time produced a similar effect.<sup>45</sup> High doses of budesonide (1600 µg a day for 12 weeks) decrease sensitivity to methacholine by 1.4 doubling concentrations.<sup>46</sup> High-dose fluticasone (500 µg/day) via hydrofluoroalkane (HFA) pMDI for 4 weeks or 4 weeks of ciclesonide via HFA

pMDI (400 µg/day) show small changes in airway sensitivity to methacholine (0.4 and 0.8 doubling concentration shifts, respectively).<sup>47</sup> Treatment with ICS over the course of 1 year led to significant improvement in methacholine PD<sub>20</sub> (3.7 doubling doses), but the improvement after 3 months was reduced to 1 doubling dose.<sup>48</sup> Collectively, these data are somewhat equivocal. The variability in response could be due to the level of baseline airway inflammation, which, in asthmatics, influences the level of asthma control and guides the dose

**Table 5** Controllers: inhaled corticosteroid, leukotriene receptor antagonist, theophylline, and ICS/LABA combination therapies

Inhaled corticosteroid						
Agent	Device	Dose	End point	Time point	Dose shift (doubling concentrations)	Reference (year)
Budesonide	DPI	400 µg bid	PC <sub>20</sub>	4 weeks	~1	Bel et al <sup>39</sup> (1991)
Budesonide (children)	MDI (with spacer)	800 µg (single dose)	PD <sub>20</sub>	5 hours	0.1	Van Essen-Zandvliet et al <sup>43</sup> (1993)
Budesonide	DPI	800 µg bid	PC <sub>20</sub>	12 weeks	~1.4	Booms et al <sup>46</sup> (1997)
Fluticasone	HFA pMDI	500 µg/day × 4 weeks	PC <sub>20</sub>	24 hours post-last dose	2.0	Lee et al <sup>47</sup> (2004)
Budesonide	DPI	200 µg/day × 8 weeks	PC <sub>20</sub>	8 weeks	0.79	Kraan et al <sup>44</sup> (1988)
Beclomethasone dipropionate	MDI+spacer	Variable	PD <sub>20</sub>	1 year	1.1	Oga et al <sup>48</sup> (2001)
Ciclesonide	HFA pMDI	400 µg/day × 4 weeks	PC <sub>20</sub>	24 hours post-last dose	0.67	Lee et al <sup>47</sup> (2004)
Leukotriene receptor antagonists						
Montelukast	Tablet	20 mg (single dose)	PC <sub>15</sub>	3 hours	~0	Crimi et al <sup>51</sup> (2003)
Montelukast	Tablet	10 mg (single dose)	PC <sub>20</sub>	1 hours	~0.4	Davis and Cockcroft <sup>50</sup> (2005)
Pranlukast	Tablet	225 mg bid × 1 week	PC <sub>20</sub>	3–4 hours post-last dose	0.68	Fujimura et al <sup>52</sup> (1993)
Theophylline						
Theophylline	IV	5 mg/L (Plasma concentration)	PC <sub>20</sub>	1 hour	0.36	Koeter et al <sup>42</sup> (1989)
		10			0.74	
		15			0.61	
Theophylline	Tablet	250 mg/day median dose	PC <sub>20</sub>	2 months	1.7	Page et al (1998) <sup>41</sup>
ICS/LABA combination treatments						
Fluticasone + formoterol	pMDI	125 + 5 bid	PD <sub>20</sub>	6 weeks	3.4	Cortese et al <sup>59</sup> (2016)
Fluticasone + formoterol	pMDI	125 bid + 12 prn	PD <sub>20</sub>	6 weeks	1.8	Cortese et al <sup>59</sup> (2016)
Fluticasone + formoterol	pMDI	250 bid + 12 prn	PD <sub>20</sub>	6 weeks	2.7	Cortese et al <sup>59</sup> (2016)

**Abbreviations:** ICS, inhaled corticosteroids; LABA, long-acting beta agonists; MDI, metered dose inhaler; pMDI, pressurized metered dose inhaler; DPI, dry powder inhaler; HFA, hydrofluoroalkane; bid, twice a day; IV, intravenous; prn, as needed; PD<sub>20</sub>, provocation dose that results in a 20% fall in the forced expiratory volume in 1 second; PC<sub>20</sub>, provocation concentration that results in a 20% fall in the forced expiratory volume in 1 second; PC<sub>15</sub>, provocation concentration that results in a 15% fall in the forced expiratory volume in 1 second.

of ICS. Treatment (non)compliance/(non)adherence may also play a role. Controller data are summarized in Table 5.

A few studies have addressed the effect of regular ICS use on other aspects of the dose–response curve; both budesonide<sup>45</sup> and fluticasone<sup>49</sup> result in the appearance or improvement in the plateau response to methacholine.

## Leukotriene receptor antagonists

Leukotriene receptor antagonists (LTRAs) block the effects of cysteinyl leukotrienes, which are potent bronchoconstricting mediators released from inflammatory cells. Anti-inflammatory effects have also been proposed. Significant inhibition of ASM contraction to leukotriene stimulation would be expected but as an add-on controller medication in the armamentarium of respiratory treatments, the effect

on MIB should be minimal or similar to ICS. Two studies have evaluated the acute effects. One study used a single oral dose of 10 mg and assessed the response at 1 hour post-dose. The other study assessed a single 20 mg dose of oral montelukast at 3 hours. Neither study showed any acute effect of montelukast on MCT outcomes.<sup>50,51</sup> Pranlukast, given orally for 1 week (550 mg daily dose), produced a similar dose shift to that of ICS (0.68 doubling concentration).<sup>52</sup> LTRA data are summarized in Table 5. Montelukast used regularly appears not to result in a methacholine dose–response plateau.<sup>53</sup>

## Antihistamines

Antihistamines are the standard of care in the treatment of allergic rhinitis. It is not uncommon for those diagnosed



with allergic rhinitis to also be diagnosed with asthma. Numerous over-the-counter antihistamines are available and examples include diphenhydramine, cetirizine, and desloratadine.

Early concerns regarding the inhibitory effect of antihistamines on bronchoprovocation with nonspecific stimuli were twofold. First, challenges were formerly conducted using histamine and second, early generation antihistamines were not necessarily devoid of anticholinergic properties. There are numerous studies in which many antihistamines have been shown to have little or no effect on MCT and this is independent of dose<sup>54–58</sup> (Table 6).

## Combination therapies

Numerous combination formulations have evolved for the treatment of respiratory disease including SAMA/SABA (eg, IB + salbutamol), ICS/LABA (eg, budesonide + formoterol), and more recently LAMA/uLABA (eg, glycopyrronium + indacaterol). Only two studies relating to the use of combination therapy and MIB could be identified. One investigation used an ICS/LABA combination and the other a LAMA/uLABA combination. The ICS/LABA combination study investigated fluticasone propionate and formoterol fumarate from a single device at a dose of 125 µg/5 µg twice per day for 6 weeks followed by prn formoterol for 4 weeks. This was shown to improve methacholine PD<sub>20</sub> by 3.4 doubling doses, although the methodology may be confounding the outcome and the result could

be the influence of formoterol alone<sup>59</sup> (Table 6). The other study used single-dose monotherapies of glycopyrronium (50 µg) and indacaterol (75 µg) administered together and measured methacholine PC<sub>20</sub> at three time points post-dose (1 hour, 24 hours, and 48 hours). At 1 hour, the combination shifted methacholine PC<sub>20</sub> by 5 doubling concentrations. At both 24 and 48 hours post-dose, bronchoprotection had decreased to 2 doubling concentrations, and this remained statistically significant (Table 2).<sup>22</sup>

## Conclusion

MCT, although mainly used as a diagnostic aid, has additional clinical applications and various research applications. The varied uses of the MCT warrant the need for understanding the effects of different respiratory medications on the outcome of the test, and this will help guide the appropriate washout periods from treatments that inhibit the response. The bronchodilator treatments that block ASM contraction either by receptor antagonism or functional antagonism are the most potent inhibitors of MIB. Depending on the agent used, the required washout period could be as soon as 6 hours or may require up to 7 days (Table 7). Controller treatments like ICS show varied efficacy, and this is probably a functional modification of the underlying airway inflammation. Combination treatments do not appear to act in an additive or synergistic way, and washout should be consistent with the monotherapy providing the longest duration of efficacy against MIB. Antihistamines do not inhibit the test.

**Table 6** Anti-H1 histamines

Agent	Route of administration	Dose	End point	Time point (hours)	Dose shift (doubling concentrations)	Reference (year)
Clemastine	Nebulized	1 mg	sGaw	0.5	nsd	Nogradyand Bevan <sup>54</sup> (1978)
Clemastine	Tablet	1 mg	PC <sub>20</sub>	4	0.82	Wood-Baker and Holgate <sup>56</sup> (1993)
Cetirizine	Tablet	20 mg	PC <sub>20</sub>	2 weeks	–0	Finnerty et al <sup>55</sup> (1990)
Cetirizine	Tablet	10 mg	PC <sub>20</sub>	2	–0	Cockcroft et al <sup>58</sup> (2015)
Cetirizine	Tablet	10 mg	PC <sub>20</sub>	2	0.26	Wood-Baker and Holgate <sup>56</sup> (1993)
Diphenhydramine	Tablet	50 mg	PC <sub>20</sub>	2	–0	Cockcroft et al <sup>58</sup> (2015)
Brompheniramine	Tablet	4 mg	PC <sub>20</sub>	4	0.59	Wood-Baker and Holgate <sup>56</sup> (1993)
Loratadine	Tablet	10 mg qd × 3 days	PC <sub>20</sub>	3	–0	Town and Holgate <sup>57</sup> (1990)
Loratadine	Tablet	20 mg qd × 3 days	PC <sub>20</sub>	3	–0	Town and Holgate <sup>57</sup> (1990)
Chlorpheniramine	Inhaled	5 mg in 3mL saline	PD <sub>20</sub>	0.5	–0.35	Woenne et al <sup>13</sup> (1978)
Chlorpheniramine	Tablet	4 mg	PC <sub>20</sub>	2	0.85	Wood-Baker and Holgate <sup>56</sup> (1993)
Terfenadine	Tablet	60 mg	PC <sub>20</sub>	2	0.09	Wood-Baker and Holgate <sup>56</sup> (1993)
Cyproheptadine	Tablet	4 mg	PC <sub>20</sub>	4	0.45	Wood-Baker and Holgate <sup>56</sup> (1993)
Astemizole	Tablet	10 mg	PC <sub>20</sub>	2	0.55	Wood-Baker and Holgate <sup>56</sup> (1993)
Desloratadine	Tablet	5 mg	PC <sub>20</sub>	2	–0	Cockcroft et al <sup>58</sup> (2015)

**Abbreviations:** qd, every day; PD<sub>20</sub>, provocation dose that results in a 20% fall in the forced expiratory volume in 1 second; PC<sub>20</sub>, provocation concentration that results in a 20% fall in the forced expiratory volume in 1 second; sGaw, airway conductance; nsd, not significantly different.

**Table 7** Recommended washout intervals prior to MCT

Drug type	Example	Washout interval
Muscarinic antagonists	SAMA (eg, ipratropium)	12 hours
	LAMA (eg, tiotropium)	7 days
Beta agonists	SABA (eg, salbutamol)	6 hours
	LABA (eg, salmeterol)	24 hours
	uLABA (eg, olodaterol)	48 hours
Xanthines	Theophylline	Not necessary
Inhaled glucocorticosteroid	Single dose (eg, budesonide)	Not necessary
	Stable dose (eg, budesonide)	Unknown
Leukotriene receptor antagonists	Single dose or up to 1 week (eg, montelukast)	Not necessary
	Stable dose (eg, diphenhydramine, desloratadine)	Unknown
Antihistamines	(eg, diphenhydramine, desloratadine)	Not necessary
	Combination therapies (limited or no data)	ICS/LABA (eg, fluticasone/formoterol)
ICS/uLABA (eg, fluticasone/vilanterol)		48 hours
LAMA/LABA (eg, glycopyrronium/indacaterol)		7 days

**Abbreviations:** MCT, methacholine challenge test; SAMA, short-acting muscarinic antagonist; LAMA, long-acting muscarinic antagonist; SABA, short-acting beta agonists; LABA, long-acting beta agonists; uLABA, ultra-long-acting beta agonists; ICS, inhaled corticosteroids.

## Disclosure

The authors report no conflicts of interest in this work.

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