Occasional review

Functional antagonism: tolerance produced by inhaled β_2 agonists

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The development of tolerance to the beneficial effects of inhaled β_2 agonists has been suggested as one possible explanation for the deleterious effects of frequent use of these agents in asthma.[1] The two main beneficial pharmacological effects of inhaled β_2 agonists in asthma are bronchodilation and functional antagonism.[2] Although it has been difficult to show appreciable tolerance to the bronchodilator efficacy of these agents,[3] regular use of inhaled β_2 agonists predictably causes tolerance to the functional antagonist (bronchoprotective effect) of these drugs.[1] In this review we will discuss the topic of tolerance to the bronchoprotective effect of inhaled β_2 agonist and its potential clinical relevance.

Functional antagonism

Functional antagonism is a term used to describe the ability of a pharmacological agent to prevent bronchoconstriction non-selectively. The term "non-selective" is used to contrast this with "selective" inhibition of bronchoconstriction – for example, inhibition of cholinergic bronchoconstriction by anticholinergic drugs or inhibition of histamine bronchoconstriction by H_1 blockers. Functional antagonism is a property shared by the bronchodilator classes of medications. β_2 agonists, particularly those administered via the inhaled route, are the most potent functional antagonists in asthma.[2] It has been suggested that tests of functional antagonism might be an alternative means of assessing the clinical effect and clinical duration of effect of inhaled β_2 agonists.[2]

β_2 agonists prevent induced bronchoconstriction predominantly by an effect on airway smooth muscle β_2 receptors.[4] These are the same receptors that are responsible for bronchodilation. It has been suggested that the mechanism of bronchodilation and functional antagonism are different, based on a poor correlation between magnitude of bronchodilation and magnitude of functional antagonism. Alternatively, the difference might be due to the difference in the way in which these are measured. Bronchodilation is essentially a "closed end" scale where improvement in flow rates is limited by return to "normal" and may be near maximal at relatively low doses of β_2 agonist. By contrast, functional antagonism, as measured by dose response curves (see below), is a more "open ended" scale. Failure of correlation between these two may be merely a result of measurement differences rather than a difference in mechanisms. Inhaled β_2 agonists may have additional effects in protecting against bronchoconstriction due to stimuli which involve mast cell mediator release. β_2 agonists suppress the release of mast cell mediators and provide greater protection against mast cell stimuli such as AMP and allergen than they do against methacholine; this is probably due to an additive protective effect on mast cell and smooth muscle β receptors in the former compared with smooth muscle only for the latter.[4]

The functional antagonism effect of an inhaled β_2 agonist is assessed in the laboratory using bronchoprovocation tests performed before and after administration of the inhaled β_2 agonist.[2,4] The difference between the two tests represents the magnitude of the functional antagonism. Stimuli for bronchoprovocation can be categorised as non-sensitising (histamine, methacholine, other chemical mediators, exercise, cold air, non-isotonic aerosols, etc) or sensitising (allergens, occupational chemicals).[5] Perhaps a more useful categorisation of bronchoprovocative stimuli is that of direct and indirect.[6] Direct stimuli provoke bronchoconstriction by a direct effect on airway smooth muscle receptors. Histamine, methacholine, and, probably, arachidonic acid metabolites are examples of direct stimuli. Indirect stimuli provoke bronchoconstriction by one or more intermediate pathways such as mast cell mediator release, neurological reflexes, etc. The sensitising stimuli, the physical stimuli (exercise, cold air, non-isotonic aerosols), and a number of the chemical stimuli (AMP, β adrenergic blockers) are felt to be indirect. Since naturally occurring bronchoconstriction in asthmatic subjects is indirect (exercise, cold air, irritants, allergens, etc), the results of challenges with indirect stimuli has been hypothesised to have more clinical relevance for asthma.[6]

There are two major different methodologies for bronchoprovocation tests that lead to two different ways to measure functional antagonism. Most commonly, bronchoprovocation tests are done by administering doubling doses of the bronchoconstrictor stimulus at appropriate time intervals to establish

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either a provocation dose (PD) or a provocation concentration (PC) producing a given fall (usually 15% or 20%) in forced expiratory volume in one second (FEV_1). The terms PD_{15}, PC_{20} are used to describe these end points. The doubling "dose" can be achieved by doubling the concentrations of the stimulus (as is often done with histamine,[7] methacholine,[4 7] and other chemical mediators[4]), or by doubling the duration of exposure as is frequently done with non-isotonic aerosols.[8 9] The early asthmatic response (EAR) to allergen can also be assessed as an allergen PC_{20}.[10] When using a bronchoconstrictor stimulus for which the results are expressed as a PC_{15} or PC_{20}, functional antagonism produced by inhaled β_2 agonists is assessed by repeating the bronchoprovocation test at an appropriate time after the administration of the β_2 agonist. The PC_{15}/PC_{20} is measured again. The degree of bronchoprotection can be assessed logarithmically by calculating the change in log PC_{20}. The $\Delta\log_{10}$ PC_{20} is divided by the log of 2 (that is, $\Delta\log_{10}$ $PC_{20}/0.3$) to express the magnitude of the functional antagonism in "doubling doses" of protection.[4] An alternative means of expressing the shift in PC_{20} is non-logarithmically as, for example, fivefold, tenfold, etc.[11] Repeating an allergen challenge to measure PC_{20} after an inhaled β_2 agonist can lead to a severe late asthmatic response (LAR).[12] When examining the allergen-induced EAR in this way, we routinely administer a single large dose of inhaled corticosteroid (beclomethasone dipropionate 500–1000 μg or equivalent) after completion of the EAR; this is known to inhibit the LAR.[13]

The second major bronchoprovocation method involves administration of a single maximal or near maximal stimulus. This is the traditional way in which exercise challenges have been done.[14] Functional antagonism is assessed by repeating the "same dose" challenge after inhaled β_2 agonist and is expressed as the percentage reduction in the response – for example, exercise induced bronchoconstriction (EIB) is usually measured as a fall in FEV_1 – compared with the response to exercise following placebo.[14 15] Functional antagonism is expressed as "percentage inhibition" by the following formula:

$$\%\ \text{inhibition} = \frac{(\text{EIB after placebo} - \text{EIB after drug})}{\text{EIB after placebo}} \times 100$$

The effect of pharmacological agents on the allergen induced LAR is also determined using a "same dose" challenge; this method is also often used when studying the EAR.

Conventional β_2 agonists and tolerance

Tolerance to the functional antagonism effect of inhaled β_2 agonists has generally been assessed 10 hours (salbutamol, terbutaline) to 36 hours (salmeterol) after discontinuing a 1–5 week course of the agent. This is compared with the functional antagonism measured before the β_2 agonist treatment period[4 11 15] or after a cross-over blinded placebo period.[10 16] The percentage change in functional antagonism, as calculated in table 1, can be expressed by comparing the shift in doubling doses (dd) before and after dosing by the formula:

$$\frac{[\text{dd (pre)} - \text{dd (post)}]}{\text{dd (pre)}} \times 100$$

For the same dose challenges – for example, exercise – this is expressed as:

$$\frac{\Delta\%\ \text{inhibition (pre} - \text{post)}}{\%\ \text{inhibition (pre)}} \times 100$$

Tolerance to the functional antagonist effect of β_2 agonists of conventional duration has routinely been found after their regular use. Gibson *et al* demonstrated that regular use of ingested salbutamol (4 mg four times daily for 2–5 weeks) in six adult subjects resulted in tolerance to the acute bronchoprotective effect of inhaled salbutamol against exercise induced bronchospasm. Protection was 78% before and 54% after treatment.[15] In the same study, inhaled salbutamol 800 μg per day in six adolescents resulted in a slight and non-significant loss of the bronchoprotective effect against exercise.[15] Vathenen *et al* studied airway responsiveness to histamine and regular use of inhaled terbutaline 750 μg three times daily for two weeks. This study demonstrated rebound airway hyperresponsiveness to histamine maximal 23 hours after stopping treatment, and a substantial loss of bronchoprotective effect of terbutaline against histamine which was greater in the afternoon than in the morning.[16] A similar study from the same group examined broxaterol 400 μg three times daily and salbutamol 200 μg three times daily for three weeks. Similar results were seen with rebound histamine hyperresponsiveness maximal at 35 hours and lasting for up to 59 hours.[17] There was less improvement in histamine PD_{20} both in the morning and in the afternoon after the three week treatment period. Salbutamol initially shifted the histamine PD_{20} by about three doubling doses and, after completion of the study, this was reduced to a little over two doubling doses. Broxaterol showed less protection at all times. This approximate 25% reduction in bronchoprotection of salbutamol against histamine was not statistically significant in these 11 asthmatic subjects. O'Connor *et al* assessed airway responsiveness to methacholine and AMP before and after one week of treatment with terbutaline 500 μg four times daily in 12 subjects.[4] Their study design did not include re-establishing the baseline PC_{20} values after each treatment. The methacholine and AMP PC_{20} values were determined following a one week treatment period with terbutaline or placebo and the PC_{20} value after a week of terbutaline treatment was compared with the baseline PC_{20} value following a week of placebo. Terbutaline initially produced a 2.7 doubling dose shift of methacholine PC_{20} compared with a 3.8 doubling dose shift of the AMP PC_{20}

Table 1 Functional antagonism before and after regular use of β_2 agonists

β_2 agonist	Stimulus	n	Duration	Functional antagonism†				Ref
				Time	Pre	Post	% loss	
Salbutamol (po)	Exercise	6	2–5 wk	10 min	~78%	~54%	~30% (*)	15
(inhaled)	Exercise	6	2–5 wk	10 min	~95%	~82%	~13% (NS)	15
Terbutaline	Histamine	8	2 wk	2 h am	~2.9 dd	~1.7 dd	40% (**)	16
				2 h pm	~3.0 dd	~0.5 dd	82% (**)	16
Salbutamol	Histamine	11	3 wk	1 h am	~3 dd	~2 dd	33% (NS)	17
				1 h pm	~4 dd	~3 dd	25% (NS)	17
Broxaterol	Histamine	11	3 wk	1 h am	~2 dd	~1.5 dd	25% (NS)	17
				1 h pm	~2 dd	~1.5 dd	25% (NS)	17
Terbutaline	Methacholine	12	1 wk	20 min	2.7 dd	2.2 dd	19% (*)	4
	AMP	12	1 wk	20 min	3.8 dd	1.7 dd	55% (**)	4
Salbutamol	Methacholine	12	2 wk	10 min	3.2 dd	2.6 dd	22% (*)	10
	Allergen	11	2 wk	10 min	3.7 dd	2.5 dd	27% (*)	10
Salbutamol (alone)	Methacholine	13	1 wk	10 min	3.0 dd	2.0 dd	33% (**)	18
(with budesonide)				10 min	2.9 dd	2.2 dd	24% (**)	18
Salbutamol								
200 µg/day	Methacholine	10	1 wk	10 min	3.4 dd	2.6 dd	24% (**)	19
400 µg/day				10 min		2.6 dd	24% (**)	19
800 µg/day				10 min		2.5 dd	26% (**)	19
Salbutamol	Exercise	10	1 wk	5 min	94%	85%	10% (?)	22
Salmeterol	Methacholine	12	4 wk	1 h	3.3 dd	1.0 dd	70% (**)	11
			8 wk	1 h		1.0 dd	70% (**)	11
Salmeterol	Methacholine	15	1 mo	12 h	1.7 dd	0.7 dd	59% (**)	25
			2 mo	12 h		1.2 dd	29% (**)	25
			3 mo	12 h		0.7 dd	59% (**)	25
			4 mo	12 h		0.8 dd	53% (**)	25
Salmeterol	Exercise	12	4 wk	6 h	66%	27%	54%	26
				12 h	40%	17%	58%	26
Salmeterol	Allergen	8	1 wk	1 h	86%	13%	85%	27
Salmeterol	Methacholine	12	3.5 days	1 h	3.3 dd	1.6 dd	52% (**)	28
				24 h††	3.7 dd	1.9 dd	53% (**)	28
Salmeterol and inhaled corticosteroid	Methacholine	8	3.5 days	1 h	2.8 dd	1.6 dd	43% (**)	29
				24 h††	2.6 dd	1.7 dd	35% (**)	29
Formoterol	Methacholine	17	2 wk	12 h	1.9 dd	0.6 dd	68% (*)	30
Salmeterol	Methacholine	10	4 wk	12 h	0.9 dd	1.2 dd	(NS)	31
			8 wk	12 h		0.7 dd	(NS)	31

† Functional antagonism: Time = timing of assessment of functional antagonism; Pre = before regular use of β_2 agonist; % = % protection in "same dose" challenges; dd = doubling dose shift of PC_{20}, PC_{15}, PD_{20}, etc; ~ = approximate (extrapolated from a graph); †† = 24 h after salmeterol completed, tested 10 min post-salbutamol. * $p<0.05$, ** $p<0.01$.

($p<0.001$). This difference was attributed to the combined effect of terbutaline on mast cell β_2 receptors and smooth muscle β_2 receptors in protecting against AMP, and on smooth muscle β_2 receptors only in protecting against methacholine.[4] After the one week treatment period terbutaline shifted the methacholine PC_{20} by 2.2 doubling doses; this 19% loss of protection was significant ($p<0.05$). By contrast, there was a much greater loss of bronchoprotective effect against AMP as terbutaline treatment only shifted the AMP PC_{20} by 1.7 doubling doses, a reduction of 55% from the 3.8 doubling doses ($p<0.001$). The interpretation of this greater loss of protection against inhaled AMP, a mast cell stimulus, was that β_2 receptors on mast cells might be more susceptible to tolerance than are the β_2 receptors on smooth muscle.[4]

Using this study as a model we investigated the effect of a two week course of salbutamol 200 µg four times daily on airway responsiveness to methacholine ($n=12$) and allergen ($n=11$).[10] The study design was different from that of O'Connor *et al* in that we re-established methacholine and allergen PC_{20} baseline values following each treatment period. We found that the methacholine PC_{20} baseline did not change, but the initial protection of 3.2 doubling doses was reduced to 2.5 doubling doses after two weeks of salbutamol ($p=0.026$), a 22% loss of protection. The protection against allergen was initially 3.7 doubling doses (greater than the protection against methacholine but not significant) and it fell by 32% to 2.5 doubling doses after the two week treatment period ($p=0.025$). The most striking finding in this study was the unexpected observation of a highly significant increase in airway responsiveness to allergen after two weeks of treatment with salbutamol. There was almost a doubling of airway responsiveness to allergen ($p=0.0009$).[10] In a follow up study we showed that salbutamol 200 µg four times a day for seven days resulted in a loss of bronchoprotection against methacholine from 2.9 doubling doses initially to 2.0 doubling doses (31% loss) after one week of salbutamol.

In the same subjects, in a blinded crossover trial, high dose budesonide (1600 µg per day) was compared with high dose budesonide plus salbutamol. An equivalent tolerance to bronchoprotection was found, with the initial protection falling from 2.9 doubling doses to 2.25 doubling doses, a 22% loss, not significantly different from the comparison between placebo and salbutamol. In this same study, although budesonide did improve the allergen PC_{15}, it did not prevent the parallel increase in airway responsiveness to allergen when salbutamol and budesonide were administered concurrently.[18] We have investigated these two models in a double blind randomised crossover study, comparing placebo with salbutamol in doses of 200 µg, 400 µg, and 800 µg per day each for one week in 10 subjects. Salbutamol produced a 3.4 doubling dose improvement in methacholine PC_{20} after placebo compared with a 2.6 doubling dose improvement following the 200 and 400 µg per day treatment periods, and a 2.5 doubling dose improvement following

the 800 μg per day treatment period.[19] This represents a 24–26% loss of the functional antagonism effect, which was not significantly different between the three doses of salbutamol. In the same study airway responsiveness to allergen did not change after 200 and 400 μg salbutamol per day for a week but did increase significantly, again by almost twofold, after 800 μg salbutamol per day for a week.[18] In two additional studies which did not assess the functional antagonist effect, the late asthmatic response to allergen was shown to be almost double after a week of regular salbutamol treatment compared with placebo; these studies were both done using the "same dose" allergen challenge model.[20 21]

Inman *et al* studied the effect of salbutamol 200 μg four times daily and exercise. Following salbutamol there was a lower FEV_1 and a lower FEV_1 after exercise, both with and without salbutamol used immediately before exercise.[22] The protection against exercise induced bronchoconstriction was excellent both after a week of placebo (94% protection) and a week of salbutamol (85% protection).

Long acting β_2 agonists and tolerance

Tolerance has also been found with the long acting inhaled β_2 agonists, salmeterol and formoterol. After regular use of either of these agents it is possible to demonstrate tolerance to bronchodilation as shown by a shift of the β_2 agonist dose–response curve to the right without a loss of maximum bronchodilation.[23 24] Tolerance to functional antagonism, however, appears to be greater than tolerance to bronchodilation.

Cheung *et al* performed a parallel placebo controlled trial of salmeterol 50 μg twice daily for eight weeks with 12 subjects receiving placebo and 12 receiving active drug. Following the first dose of salmeterol there was a tenfold improvement in methacholine PC_{20} (3.3 doubling doses). After a 36 hour washout period at four and eight weeks there was no change in FEV_1, no change in bronchodilation, but a dose of salmeterol at these two time periods offered only a twofold improvement in methacholine PC_{20} (that is, one doubling dose). This represents approximately a 70% loss of bronchoprotective effect. In this study there was no rebound hyperresponsiveness after the eight week treatment was discontinued.[11] In a recent parallel study in children of salmeterol 50 μg twice daily ($n=15$) versus salbutamol 200 μg twice daily ($n=15$) similar results were seen.[25] Twelve hours after the first dose of salmeterol the methacholine PD_{20} increased by 1.7 doubling doses. After 1–4 months the methacholine PD_{20} was shifted, after a dose of salmeterol, by 0.7, 1.15, 0.65, 0.76 doubling doses at one, two, three, and four months, respectively; these were significantly less than the protection after the first dose ($p<0.001$). Methacholine PD_{20} measured 12 hours after salbutamol in the parallel group showed no changes at any time.

Ramage *et al* studied the airway response to exercise six and 12 hours after the first and last dose of salmeterol, 50 μg twice daily for four weeks. Since exercise was studied, a "same dose" challenge was used. The first dose of salmeterol produced a 66% reduction in the exercise induced fall in FEV_1 at six hours and a 40% reduction in the fall in FEV_1 at 12 hours. The last dose produced only a 27% reduction in exercise induced bronchoconstriction at six hours and an 18% reduction at 12 hours, the results with the last dose being not statistically different from those with placebo, despite a trend. These results represent a 55–60% loss of bronchoprotection.[26]

Gianinni *et al*, in a published abstract, studied eight subjects who undertook a same dose allergen challenge one hour after the first and last dose of salmeterol and placebo administered for one week in a crossover study. The first dose of salmeterol almost completely inhibited the early asthmatic response (86% inhibition), whereas the last dose of salmeterol was not significantly different (13% inhibition) from placebo. This represents a 77% loss of bronchoprotection.[27]

We have studied the time course of the development of tolerance to the bronchoprotective effect of salmeterol against methacholine. Methacholine PC_{20} was measured one hour after the first, third, fifth, and seventh doses of twice daily salmeterol in a double blind placebo crossover study. Twenty four hours after salmeterol was discontinued the methacholine PC_{20} was measured 10 minutes after 200 μg inhaled salbutamol. By the third dose of salmeterol the bronchoprotective effect was reduced from 3.3 to 2.4 doubling doses ($p=0.009$) and continued to fall to a protection of only 1.5 doubling doses after the seventh dose. The PC_{20} after salbutamol on day 5 was also reduced from a 3.7 doubling dose shift 24 hours after the seventh dose of placebo to 1.9 doubling dose shift 24 hours after the seventh dose of salmeterol.[28] Similar findings were reported in a subsequent study of identical design in eight subjects with more severe asthma who required regular inhaled corticosteroids.[29] The initial dose of salmeterol provided an improvement of approximately three doubling doses in methacholine PC_{20}, while the last dose provided less than 1.5 doubling dose protection. The loss of protection was again significant by the second day ($p=0.031$) and again extended to salbutamol given 24 hours after the last dose.

Yates *et al* assessed methacholine PC_{20} after the first and last dose of a two week treatment period of formoterol 24 μg twice daily. Twelve hours after the first dose of formoterol there was a 1.9 doubling dose improvement in methacholine PC_{20} compared with a 0.5 doubling dose increase 12 hours after the last dose. This represents a 74% loss of bronchoprotection. This study also failed to show any significant rebound hyperresponsiveness at 36 hours, 60 hours, 108 hours, and two weeks after the two week course of formoterol.[30] The FEV_1 was lower 36 hours after formoterol than after placebo, but was not lower at other time periods.

The one contradictory study published was that by Booth *et al* who studied 26 asthmatic

patients, 19 of whom were on maintenance inhaled corticosteroids. They were allowed free access to inhaled β_2 agonist before and during the trial as "rescue" medication, so any tolerance may have been present at the beginning of the study. The study was a parallel, double blind, eight week trial with 10 subjects completing the salmeterol arm and 12 the placebo arm. Methacholine PC_{20} was measured 12 hours after the first dose and repeated 12 hours after a dose at four and eight weeks. The functional antagonism effect after the first dose was less than one doubling concentration of methacholine and there was no loss over time.[31] It is likely that the negative results in this trial were caused by the pre-trial (and continued) use of rescue inhaled β_2 agonist.

The results of all of the trials quoted are summarised in table 1. Whereas the short acting β_2 agonists generally appear to result in a 20–30% loss of bronchoprotection, the longer acting β_2 agonists consistently seem to cause a 50–75% loss of bronchoprotection. Although still difficult to demonstrate tolerance to bronchodilation, this is easier for the longer acting than for the conventional β_2 agonists.[23 24]

Conclusions

The published studies are very consistent. Tolerance to the functional antagonist effect of inhaled β_2 agonists develops with all agents and to all stimuli (table 1). Significant tolerance can be seen at very low doses (salbutamol 200 μg per day for seven days)[19] and can develop very rapidly (by the third dose of twice daily salmeterol).[28 29] Corticosteroids do not prevent development of this tolerance.[18 29] The mast cell stimuli (AMP, allergen, possibly exercise) appear to be more prone to tolerance than are the direct chemical stimuli.[4] In addition to tolerance to the bronchoprotective effect, regular use of inhaled β_2 agonists increases baseline airway responsiveness to allergens – both early[10 18 19] and late[20 21] – such that the post-salbutamol allergen PC_{20} after two weeks of salbutamol was almost two doubling doses lower than after placebo.[10]

The clinical relevance of these findings remains uncertain. Even when tolerance develops, particularly with the short acting inhaled β_2 agonists, there remains a considerable functional antagonist effect. However, inhaled β_2 agonists are often used clinically for their functional antagonist effect – for example, before exercise, exposure to cold air, and exposure to allergens. It therefore seems likely that the important loss of bronchoprotection against allergen and exercise seen particularly with the long acting inhaled β_2 agonists might have important clinical relevance. The enhanced airway response to allergen, particularly the late response, may be associated with increased allergen induced airway inflammation.[21] This may explain the observation of Manolitsas *et al* of increased levels of eosinophils in bronchial biopsy specimens taken after regular treatment with salbutamol.[32] These findings may also explain the appearance of increased airway responsiveness (or rebound airway responsiveness) seen in some studies[16 17 33 34] but not in others.[10 11 18 19 30]

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