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Past, present and future—β_2-adrenoceptor agonists in asthma management

Malcolm R. Sears[a,*], Jan Lötvall[b]

[a]*Department of Medicine, McMaster University and Firestone Institute for Respiratory Health, St Joseph's Hospital, 50 Charlton Avenue East, Hamilton, Ont., L8N 4A6 Canada*
[b]*The Lung Pharmacology Group, Department of Respiratory Medicine and Allergology, Göteborg University, Sahlgrenska University Hospital, Bruna Stråket 11, S-413 45 Göteberg, Sweden*

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Summary The β-adrenoceptor agonists (β-agonists) have been used to relieve bronchoconstriction for at least 5000 years. β-agonists are based on adrenaline and early forms, such as isoprenaline, lacked bronchial selectivity and had unpleasant side effects. Modern β-agonists are more selective for the β_2-adrenoceptors (β_2-receptors) located in bronchial smooth muscle and have less cardiotoxicity. Traditional β_2-adrenoceptor agonists (β_2-agonists), such as salbutamol, terbutaline and fenoterol, were characterised by a rapid onset but relatively short duration of action. While valuable as reliever medication, their short duration gave inadequate night-time relief and limited protection from exercise-induced bronchoconstriction. β_2-agonists with longer durations of action, formoterol and salmeterol, were subsequently discovered or developed. When combined with inhaled corticosteroids they improved lung function, and reduced symptoms and exacerbations more than an increased dose of corticosteroids. However, tolerance to the bronchprotective effects of long-acting β_2-agonists and cross-tolerance to the bronchodilator effects of short-acting β_2-agonists is apparent despite use of inhaled corticosteroids. The role of β_2-receptor polymorphisms in the development of tolerance has yet to be fully determined.

Formoterol is unique in having both a long-lasting bronchodilator effect (>12 h) and a fast onset of action (1–3 min from inhalation), making it effective both as maintenance and reliever medication. The recent change in classification from short- and long-acting β_2-agonists to rapid-acting and/or long-acting agents reflects the ongoing evolution of β_2-agonist therapy.

*Corresponding author. Tel.: +1-905-522-1155x3286; fax: +1-905-521-6132.
E-mail address: searsm@mcmaster.ca (M.R. Sears).

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Introduction

Asthma is a serious public health problem, being one of the most common diseases in industrialised countries. There is convincing evidence that the prevalence and morbidity of asthma are increasing.[1,2] In the USA, 14 million adults and 5.2 million children were affected by asthma in the mid-1990s—an increase from the 1980 figures of 6.7 million and 4 million, respectively.[3] Costs related to asthma were estimated to reach $14.5 billion in the USA by the year 2000.[3] 1ncreases such as these have led to greater awareness of the importance of patient and physician education—especially regarding the risk factors for asthma and the treatment strategies available.

Since inflammation of the airway tissues is considered to be the main mechanism in the development and maintenance of asthma, limiting exposure to inflammatory triggers and reducing the inflammatory process using anti-inflammatory drugs are the main thrusts of the modern management of asthma.[4,5] The first-line anti-inflammatory drugs, inhaled corticosteroids (ICS), may be adequate to fully control symptoms in mild cases. However, for many patients, additional drug therapy, typically bronchodilators that relax airway smooth muscle, is needed for the relief of acute symptoms.[6] β_2-adrenoceptor agonist bronchodilators (β_2-agonists) are the most effective and widely used drugs to produce rapid reversal of bronchoconstriction. Although traditionally classified by their duration of action, as short-acting and long-acting β_2-agonists, such classifications may not now reflect the full therapeutic relevance of these agents.

β-adrenoceptor agonists (β-agonists), one of the oldest classes of drugs used in medicine, act by mimicking the effects of adrenaline. Sympathomimetic agents were used in Chinese herbal medicines to relieve breathing difficulties as early as 3000 BC. The active material, an alkaloid now identified as ephedrine, was originally extracted from the plant *Ephedra equisetina* and known as Ma Huang.[7] However, ephedrine was only introduced into western medicine as recently as 1924.[8] The β_2-agonists have become standard bronchodilators for emergency room treatment of asthma, and as day-to-day reliever medication. Their role in maintenance treatment to control asthma in conjunction with other medications, particularly ICS, has been re-evaluated over the past decade. This paper is a non-systematic review of the evolution of β-agonists and provides a perspective on their current role in the management of asthma.

Development of non-selective β-agonists

Adrenaline

Modern sympathomimetic drugs derive from the discovery of a bronchodilating 'adrenal substance' by Solis-Cohen in 1900[9] and the joint isolation of the active agent, adrenaline, by John Abel in 1899 and by Jokichi Takamine in 1901. By relaxing bronchial smooth muscle, in preparation for the increased ventilation needed for 'flight or fight', endogenous adrenaline offers a potent and focussed treatment for the breathing difficulties associated with asthma.

Adrenaline (epinephrine; Fig. 1)[10] was initially given parenterally, then by aerosol, and was widely used in the treatment of asthma for at least 35 years following its discovery. However, although inhaled adrenaline gave patients rapid relief of asthma symptoms, concerns arose about possible cardiotoxicity and the development of tolerance.[11] The overuse of nebulised adrenaline spray that resulted from tolerance was linked with a fivefold increase in mortality in patients with asthma who used the spray compared with non-users.[11] Thus, there was a need for drugs with the bronchodilating effect of adrenaline but which had greater selectivity for airway smooth muscle and correspondingly fewer side effects.

Isoprenaline

The first pure β-agonist to be synthesised was isoprenaline (isoproterenol; Fig. 1), developed in the 1940s. Although an effective bronchodilator and more selective than adrenaline,[12] isoprenaline was still associated with substantial extra-pulmonary side effects, such as palpitations, tachycardia and headache. Isoprenaline, like adrenaline, is a catecholamine, a class of drugs characterised by chemical and metabolic instability resulting in a short duration of action.[13]

The discovery that adrenaline and isoprenaline had different physiological properties led to a greater understanding of the nature of their specificity and allowed more selective drugs to be developed. Ahlquist[14] noted that adrenaline was more potent than isoprenaline in smooth muscle that responded by contraction, but the opposite was true in smooth muscle that responded by relaxation. He suggested that tissues contained different types of adrenergic receptors and that the two drugs demonstrated different properties by acting as agonists at these different receptors. This initial classification introduced the concept of

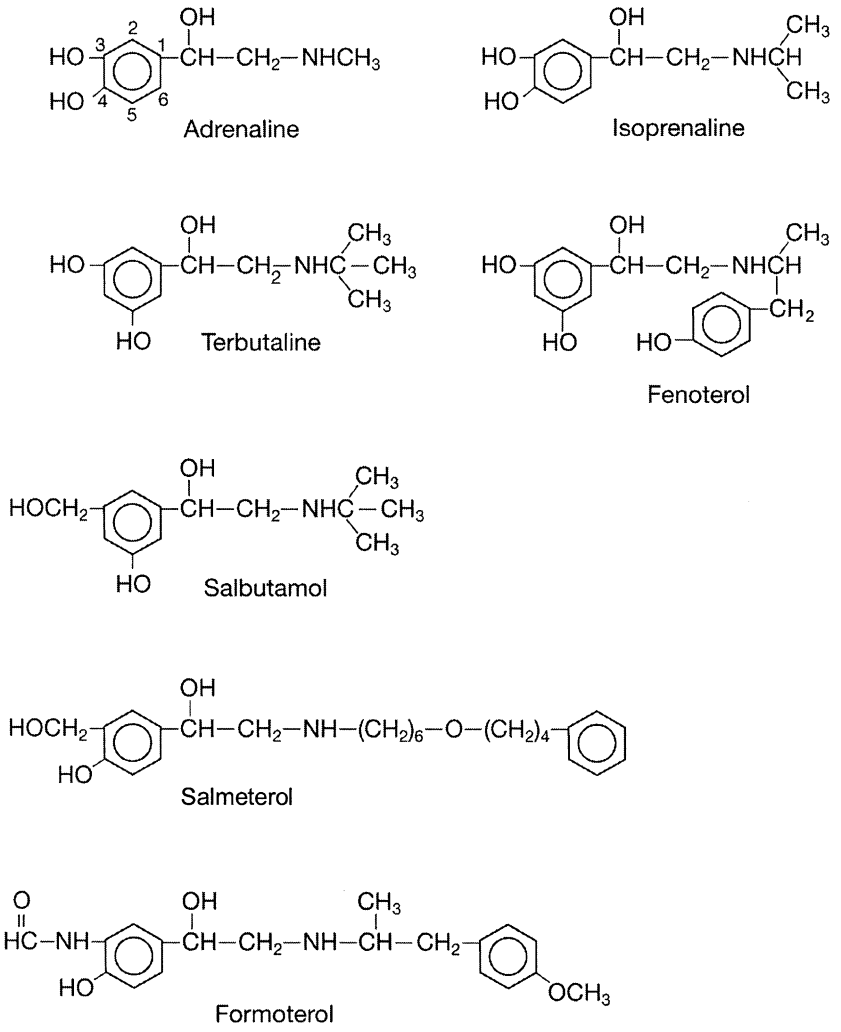


Figure 1 Chemical structure of selected β-agonists.[10] Reproduced with kind permission from the Australian Medical Association.

alpha-(α) and beta-(β) adrenoceptors, with α-receptors associated with vasoconstriction, stimulation of the uterus and intestinal relaxation, and β-receptors with vasodilation, bronchodilation and myocardial stimulation.

Although adrenaline had both α- and β-receptor agonist activity, isoprenaline had only β-receptor agonist properties. However, although this accounted for the different side-effect profiles of the two drugs, explaining why some of the side effects (such as urinary retention and hypertension) were associated only with adrenaline, it did not explain the undesirable side effects of isoprenaline.

An analogue of isoprenaline, isoetharine (1951), showed increased bronchodilator selectivity and duration of effect, but still had undesirable cardiac side effects. Only with the discovery that there was more than one type of β-receptor[15] did greater selectivity become possible. The β_1-receptor was identified in the heart and intestinal smooth muscle, while the β_2-receptor was identified primarily in bronchial, vascular and uterine smooth muscle. Isoprenaline was a non-selective β-agonist (no α-receptor activity but activity at both β_1- and β_2-receptors), which explained its side-effect profile. Isoetharine improved the benefit-to-risk ratio as a result of low α-receptor activity and enhanced β_2-receptor activity, as did the more potent and longer-acting resorcinol analogue orciprenaline (metaproterenol) introduced in the early 1970s. However, both isoetharine and metaproterenol had undesirable cardiac side effects due to action on β_1-receptors. Furthermore, the presence of a significant population of β_2-receptors in cardiac muscle meant that some cardiac stimulation was inevitable even with a totally selective β_2-agonist.[16] Use of small, inhaled doses of highly selective β_2-agonists was expected to result in preferential activation of pulmonary β_2-receptors,

with minimal side effects from the drug reaching the systemic circulation and peripheral tissues.[17] However, to target drugs more effectively, more needed to be known about the β_2-receptor and its mechanism of activation.

The β_2-receptor

The β-receptor is a glycoprotein embedded in the plasma membranes of a number of cell types. Three distinct subtypes of β-receptors are now known, β_1, β_2, β_3, found predominately in cardiac muscle, airway smooth muscle and adipose tissue, respectively.[18] The β_2-receptor is composed of 413 amino acid residues arranged in seven membrane-spanning alpha-helices.[19] These helices form a three-dimensional binding site accessible via the extracellular aqueous biophase.[20] Approximately 80% of the β-receptors in the lungs are of the β_2-subtype.[21] However, there is a homology of 54% between the human β_1- and β_2-subtypes,[22] which may result in any highly selective β_2-agonist having an effect on β_1-receptors.

Ninety percent of the β_2-receptors in the lungs are thought to be located in the alveolar wall,[23] with the remainder found on smooth muscle cells and in the membranes of epithelial, endothelial and mast cells.[24] Smooth muscle cells may each contain 30 000–40 000 β_2-receptors.[25] No difference has been reported in the number of receptors found in healthy subjects and those with asthma, although an inverse relationship has been reported between lung β_2-receptor density and forced expiratory volume in 1 s (FEV_1) (% predicted).[25] Of relevance to the occurrence of cardiac side effects, however, is that up to 40% of β-receptors in the ventricles and up to 55% in the atria are also of the β_2-subtype.[26]

β-receptor activation

β-receptor activation increases levels of intracellular cyclic adenosine monophosphate (cAMP) via G-protein activation of adenyl cyclase.[27] The cAMP is then thought to influence key regulatory proteins (cAMP-dependent protein kinases) involved in the control of muscle tone, inhibit calcium ion release from intracellular stores, reduce calcium ion entry into the cells, and sequester intracellular calcium ions.[17] The result is to bring about relaxation of the central and peripheral airway smooth muscle and hence bronchodilation. Prolonged receptor binding may however lead to desensitisation through uncoupling or sequestration[28,29] and may interfere with the action of other ligands,[30] possibly due to continued receptor occupation.

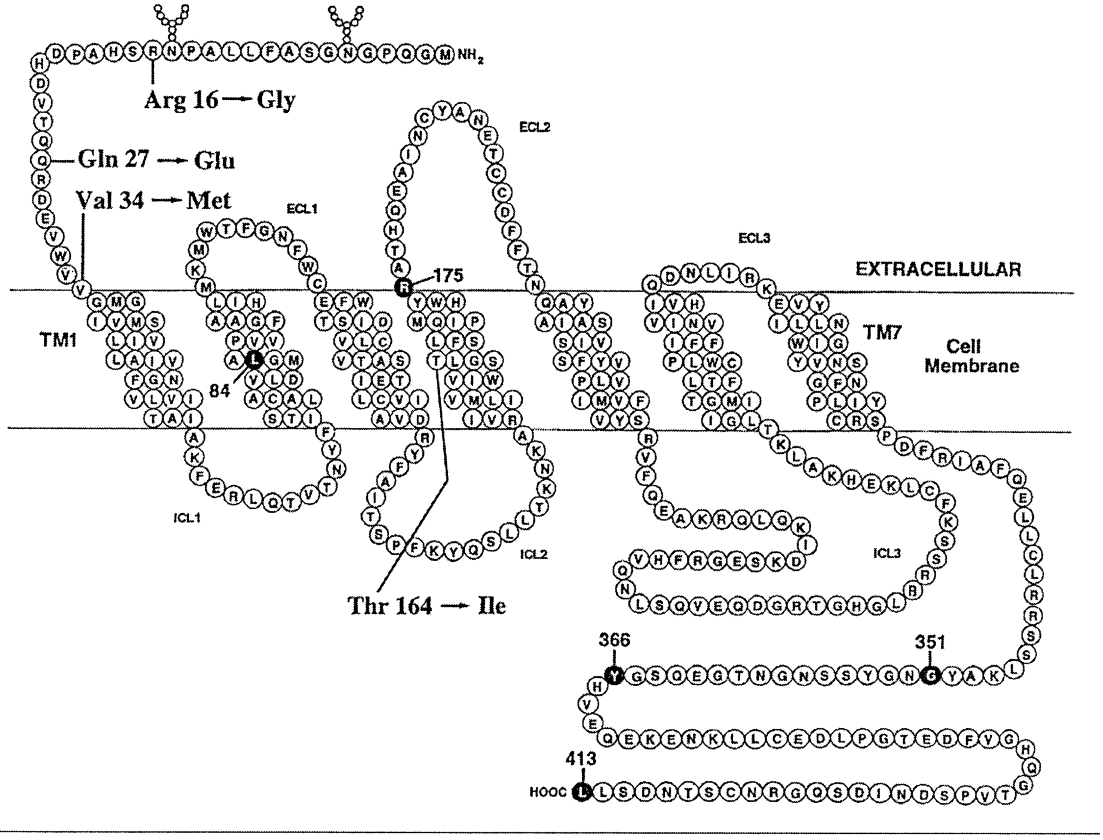
Ligands bind within the hydrophobic core of the receptor, with critical interactions taking place between chemical groups of the agonist and certain key residues.[24] The three-dimensional arrangement of these interacting chemical groups has implications for structural changes resulting from polymorphisms in the receptor, and may also influence the development of tachyphylaxis.

β-receptor polymorphisms

The gene encoding the human β_2-receptor is a gene without introns, located on the long arm of chromosome 5, and has a coding block of 1239 nucleotides.[31–33] Nine variations occur in the normal population.[31,32,34] These polymorphisms are point mutations distributed throughout the coding block and are found at nucleotide positions 46, 79, 100, 252, 491, 523, 1053, 1098 and 1239.[34] Five are degenerate and the remaining four, at nucleotides 46, 79, 100 and 491, result in single amino acid substitutions at positions 16 (arginine to glycine), 27 (glutamine to glutamate), 34 (valine to methionine) and 164 (threonine to isoleucine)[33,34] (Fig. 2). Amino acids 16 and 27 lie in the extracellular N-terminal domain while 34 and 164 are in the transmembrane spanning regions.[33]

The most common polymorphisms are at amino acid positions 16 and 27 whereas that at 34 is rare, with an allele frequency of $<1\%$.[32,35] The polymorphism at 164 is uncommon, the heterozygous state having been found in only 3–5% of the population.[32,35] The frequency of the position 16 and 27 variants differs among white, black and Asian populations.[32,36] All these polymorphisms have been found with equal frequency in both healthy individuals and patients with asthma and are thus unlikely to be the cause of asthma per se. However, they may influence the phenotype of the illness once it is expressed,[33] by acting as disease modifiers, altering baseline airway function or the response to β_2-agonists.[35]

The cellular phenotypes of these polymorphisms are shown in Table 1. The Ile164 variant shows altered agonist binding and altered receptor downregulation following agonist simulation.[37] Gly16 and Gln27 are associated with increased downregulation following agonist exposure, while Glu27 appears to be protective against downregulation. Several studies have shown an association between polymorphisms 16 and 27 and airway responsiveness. Hall et al.[38] showed that patients with asthma who were homozygous for Glu27 had less reactive



TM, transmembrane spanning domain; ECL, extracellular loop; ICL, intracellular loop.

Figure 2 Primary amino acid sequence and proposed membrane topography of the human β_2-receptor. Reprinted with permission from Blackwell Publishing.[34]

Table 1 Cellular phenotypes of β_2-adrenoceptor polymorphisms as determined in cell and transgenic systems. Reprinted with permission from the American Academy of Allergy Asthma and Immunology.[35]

Nucleic acid no.	Amino acid no.	Designation	Phenotype
46	16	Arg16	Wild type
46	16	Gly16	Enhanced downregulation
79	27	Gln27	Wild type
79	27	Glu27	Absent downregulation
100	34	Val34	Wild type
100	34	Met34	Normal
491	164	Thr164	Wild type
491	164	Ile164	Decreased coupling, binding and sequestration

airways than those who were homozygous for Gln27, showing a fourfold higher geometric mean methacholine PD_{20} (provocative dose causing a 20% fall in FEV_1). Ramsay et al.[39] also found an association between Glu27 and decreased airway responsiveness to histamine. ln addition, Arg16 was associated with increased wheeze during respiratory infection.[39]

Kotani et al. showed that patients with asthma homozygous for Gly16 had significantly ($P<0.05$) lower airway responsiveness to inhaled salbutamol than those heterozygous for Arg/Gly16 or homozygous for Arg16, and that those heterozygous for Gln/Glu27 had a significantly ($P<0.05$) later onset of asthma than those with the wild-type genes.[40] Similarly, Lima et al.[41] showed that Arg16

polymorphism is a major determinant of bronchodilator response to salbutamol: patients homozygous for Arg16 had a more rapid increase in FEV_1 and a higher bronchodilator response 1 h after salbutamol administration than patients with the Gly16 allele. In a study of 23 patients with nocturnal asthma and 22 patients with non-nocturnal asthma, the frequency of the Gly16 allele was 80% in the noctural group and 52% in the non-nocturnal group ($P = 0.007$) with an odds ratio of 3.8 for having nocturnal asthma and the Gly16 allele.[42]

Hancox et al.[43] found no association between β_2-receptor polymorphism and the deleterious response to long-term, regular fenoterol treatment. In addition, Lipworth et al.[44–46] showed in a number of studies that polymorphism at codon 16 or 27 did not influence the degree of functional antagonism exhibited by formoterol, salmeterol or terbutaline. In contrast, Taylor et al.[47] found that among patients homozygous for Arg16, the frequency of major exacerbations more than doubled during treatment with salbutamol compared with placebo and that the rate of exacerbations during salbutamol treatment was five times greater than among homozygous Gly16 patients. Israel et al.[48] also showed that polymorphism at codon 16 had an effect on the response to the regular use of salbutamol: patients homozygous for Arg16 showed a decline in morning peak expiratory flow (PEF) while those homozygous for Gly16 did not.

The Gly16 polymorphism may also determine patient susceptibility to bronchodilator desensitisation. Tan et al.[49] demonstrated a significantly ($P<0.05$) greater degree of bronchodilator desensitisation following treatment with formoterol in patients homozygous for Gly16 than in patients homozygous for Arg16. Conversely, Lee et al.[50] indicated that the Arg16 polymorphism was associated with subsensitivity of response for bronchoprotection in patients taking regular ICS. This subsensitivity was greater for formoterol than for salmeterol.[50] The different responses to formoterol and salmeterol may reflect differences in intrinsic receptor efficacy with regard to prolonged receptor occupancy.

Subsensitivity may also be influenced by endogenous catecholamines and their effect on basal β_2-receptor regulation. Liggett[35] suggested two possible models of receptor kinetics. The dynamic model indicated that the glycine form of β_2-receptor would be more susceptible to downregulation by endogenous catecholamines, whereas the arginine form would show basal upregulation, resulting in tolerance and subsensitivity upon β_2-agonist exposure. The alternate, static model suggests little or no effect of endogenous catecholamines, resulting in the glycine β_2-receptor form being more susceptible to downregulation and the arginine form less susceptible when exposed to exogenous β_2-agonists. Jackson and Lipworth[51] performed a retrospective analysis of data from six placebo-controlled, randomised, cross-over studies in an attempt to resolve which model is applicable in patients. Their results support the dynamic model, since Arg16 polymorphisms were associated with a subsensitivity of response to long-acting β_2-agonists.

Although point mutations clearly influence response to β_2-agonists, the effects of combinations of mutations into complex haplotypes must also be considered. Drysdale et al.[52] investigated response to salbutamol in 121 patients with asthma. Twelve different haplotypes were expressed within the group. For the five most common in vivo, β_2-agonist response was significantly related to the patients' genotype ($P = 0.007$). Haplotype pair 4/6 showed the greatest increase in FEV_1 while 4/4 showed the smallest response. A similar study, however, showed no significant effect of haplotype on the in vivo response to isoprenaline.[53]

The exact clinical implication of different polymorphisms of the β_2-receptor, and their impact on acute or regular treatment with β_2-agonists, is not yet fully elucidated. Further prospective and detailed studies of short- and long-acting β_2-agonists in large patient groups with different receptor genotypes are needed.

Development of more selective β_2-agonists

The total effect of any β_2-agonist involved in bronchodilation is a property of its β_2-receptor binding affinity and its ability to induce an intracellular response. The latter is due to a conformational change in the receptor leading, in turn, to one or more intracellular events. The potency of a drug is related to the amount required for a physiological response, whereas efficacy is a term related to the drug's ability to induce maximum physiological effects. Although isoprenaline had a high potency and high pharmacological efficacy (Table 2), extensive interactions with β_1-receptors limited its usefulness because of its potential to cause cardiovascular side effects. Hence, in the 1960s more selective β_2-agonists, such as fenoterol, salbutamol and terbutaline, were developed.

Fenoterol

Fenoterol (Fig. 1) is a resorcinol derivative of orciprenaline, with negligible α-receptor-stimulating

activity. It is relatively selective for β_2-receptors, although less so than salbutamol and, possibly, terbutaline.[54] Inhaled fenoterol produces superior bronchoprotection compared with isoprenaline.[54] Although the time to maximum effect is longer with fenoterol, its duration of action (4–6 h) is also longer compared with isoprenaline (Table 2) and it has less effect on heart rate.[54] ln one early study, 20 of 22 patients with asthma receiving both fenoterol and isoprenaline aerosol inhalers expressed a preference for fenoterol.[55]

Salbutamol

Salbutamol (albuterol), developed by modifying the basic catechol nucleus common to the naturally occurring adrenergic neurotransmitters adrenaline and noradrenaline, was introduced in 1969. Salbutamol is longer lasting than isoprenaline and isoetharine because it is not broken down by catechol *O*-methyltransferase.[56] The salbutamol molecule (Fig. 1) is hydrophilic and accesses the β_2-receptor directly from the aqueous extracellular compartment. This results in a fast onset of action, with effective bronchodilation occurring within 2–3 min and peak bronchodilation within 15 min of inhalation.[57] However, the molecule binds only weakly to the receptor and quickly diffuses back into the microcirculation. This accounts for its short duration of action (4–6 h), similar to that of fenoterol (Table 2).

Salbutamol has negligible α-receptor activity and demonstrated a $>$500-fold greater selectivity between β_2- and β_1-receptors than any other product previously available.[58] Salbutamol thus overcame many of the limitations of its predecessors and quickly became the most widely used and popular β_2-agonist.

Levalbuterol (*R*-albuterol), a single isomer of salbutamol, is currently available as a nebulised solution in the USA. Several studies have compared levalbuterol with salbutamol (racemic albuterol) and suggest that use of the single isomer may reduce hospitalisations,[59] have fewer adverse effects[60] and provide similar bronchodilator effects at a reduced dose.[61] However, other studies have indicated similar efficacy of the isomer and the racemic form,[62,63] echoed by the current international guidelines on the management of asthma.[64] Further development and evaluation of enantiomers may clarify whether there are significant benefits to be obtained from single isomer forms of β_2-agonists.

Terbutaline

Terbutaline (Fig. 1) is a non-catecholamine β_2-agonist related to orciprenaline. It is also resistant to degradation by catechol *O*-methyltransferase because it contains a resorcinol group.[65] lt was specifically developed to relax the trachea without affecting the cardiac muscle, and was first introduced in 1970.[66] Bronchodilation occurs rapidly after inhalation and effects may persist for 4–6 h (Table 2). Terbutaline is useful for as-needed medication. A prodrug of terbutaline, oral bambuterol, is also an effective bronchodilator when given orally, and has a longer duration of action similar to that of salmeterol.[67]

Limitations of short-acting β_2-agonists

Although terbutaline and salbutamol are very widely used, they have a relatively short duration of action. Whether taken by inhalation or by mouth, the duration of bronchodilation achieved

Table 2 Summary of some pharmacological properties of selected β_2-agonists.

	Affinity for β_2-receptor (K_i) (nM)	Efficacy at β_2-adrenoceptors (relative to isoprenaline as 100%)	Potency at β_2-adrenoceptors (relative to isoprenaline)	Selectivity ratio ($\beta_2 : \beta_1$ receptors)	Approximate onset of action (min)	Approximate duration of action
Isoprenaline	200	(100)	(1.0)	1:1	2–5	$<$20 min
Salbutamol	2500	86	0.55	1:1375	2–3	4–6 h
Fenoterol	—	100	—	1:120	2–4	4–6 h
Terbutaline	—	65–85	—	—	2–4	4–6 h
Salmeterol	53	63	8.5	1:85 000	30	$>$12 h
Formoterol	76	100	20.0	1:120	2–3	$>$12 h

Data derived from Refs.[25,58,78,141] (—denotes data not available).

with a single dose of either drug does not exceed 4–6 h. Although this does not affect their usefulness for as-needed medication, it does impose limitations for patients who require continuing bronchodilator therapy to reverse airway narrowing and long-term protection from bronchospasm, e.g. patients with nocturnal asthma. The need for β_2-agonists with a longer duration of action was the impetus for the development of formoterol and salmeterol.

β_2-agonists with long-acting properties

There are currently two inhaled β_2-agonists with durations of action in excess of 12 h, formoterol and salmeterol. Each molecule was developed to interact specifically with the β_2-receptor. Their profiles are best understood by considering their different physicochemical properties in the microenvironment of the β_2-receptor, including the cell membrane.

Formoterol

Formoterol (eformoterol in the UK) is a formanilide-substituted phenoethanolamine (Fig. 1) and was synthesised as part of a series of molecules systematically developed for increasing β_2-receptor selectivity and bronchodilator potency.[68] Although originally developed for oral use, formoterol was subsequently found to have an extended duration of action when taken by inhalation.[69]

When β_2-agonists are inhaled, high topical concentrations are instantly deposited on the airway epithelia: concentrations of formoterol and salmeterol of 1 μmol/l have been estimated in the periciliary fluid of the bronchi after a single inhalation.[20] The molecules then diffuse across the epithelium towards the airway smooth muscle. Formoterol is moderately lipophilic ($K_{\rho[mem]}$ 500 : 1)[70] and most of the inhaled dose that reaches the smooth muscle layer is taken up into the cell membranes. This forms a depot from which formoterol is thought to progressively leach out to interact with the β_2-receptors (Fig. 3). Formoterol thus has a prolonged effect, causing bronchodilation that lasts for more than 12 h[72,73] (Table 2). However, sufficient numbers of molecules remain in the aqueous phase outside the cells to allow immediate interaction with β_2-receptors and, therefore, a fast onset of bronchodilation (within 1–3 min).[74] Hence, formoterol acts as rapidly as salbutamol.[75]

Compared with salmeterol and salbutamol, formoterol is a full agonist at the β_2-receptor and results in more than 80% of maximal β_2-receptor activation.[71] In terms of relaxing isolated human bronchi, formoterol has been found to be >100 times more potent than salmeterol and >300 times more potent than salbutamol.[76]

Salmeterol

Salmeterol is a saligen derivative of phenylethanolamine (Fig. 1) developed in the early 1980s and has been available clinically since 1990. When the molecule was designed, the head group of salbutamol was used, taking advantage of this molecule's β_2-agonist properties and minimal side-effect profile on other adrenoceptors. The aliphatic side chain was extended to interact with a theoretical

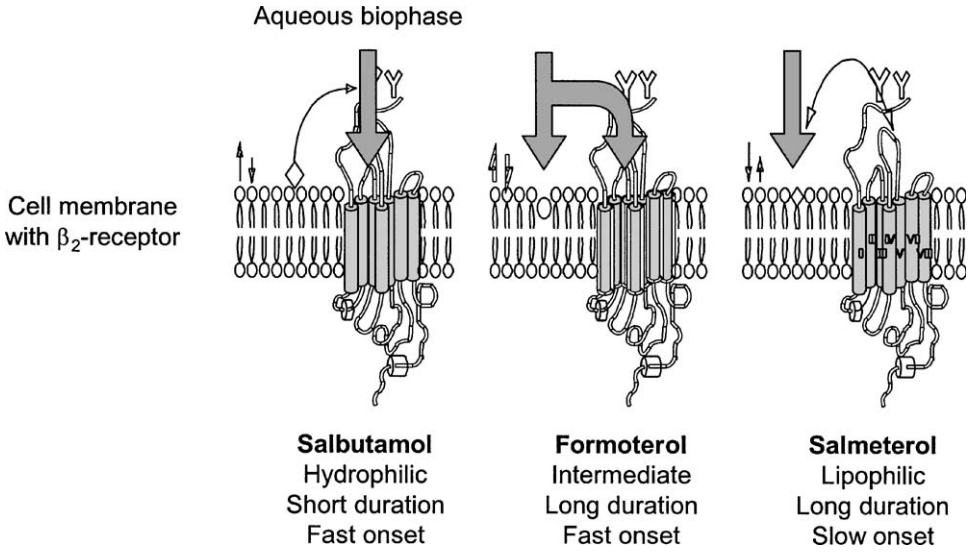


Figure 3 Diagrammatic representation of the diffusion microkinetic hypothesis. Reprinted with permission from Elsevier.[71]

exosite inside or outside of the β_2-receptor, to prolong the action of the salmeterol molecule.[68]

The salmeterol side chain has a length of 17 Å, which makes the molecule >10 000 times more lipophilic than salbutamol.[58] Salmeterol therefore partitions rapidly (within 1 min) into the outer phospholipid monolayer of any cell membrane in its vicinity ($K_{\rho[mem]}$ 22,500: 1), and appears to diffuse laterally along the cell membranes, at approximately 2 μm/s .[77] However, to diffuse through the tissue, from epithelium through the submucosa to the bronchial smooth muscle, salmeterol has to diffuse out of one cell membrane, to enter the next, to continue its diffusion. Since most salmeterol molecules are present in cell membranes, this is a relatively slower process than that observed with formoterol, thus explaining the slower onset of action of salmeterol. Furthermore, little interaction with β_2-receptors on the interstitium may also contribute to the relatively slower onset of action of salmeterol. Clinically, salmeterol does not achieve its maximum effect until approximately 30 min after inhalation, whereas formoterol, salbutamol and terbutaline reach clinically important effects within 3 min, and close to maximum effects within 10–15 min.[57,73,75]

The extended duration of action of salmeterol has been explained by its long aliphatic side chain acting via an exosite within the β_2-receptor.[24,58,78,79] Although this could explain its extended duration of action and the molecule's ability to 'reassert' its effect after multiple wash-out cycles, other molecules (including formoterol) also have this property of reassertion without having long side chains. Consequently, the exosite hypothesis is not universally accepted.[20] Pre-treatment of airway smooth muscle tissue with the pure aliphatic side chain of salmeterol failed to block the long duration of salmeterol, which should have occurred if the anchored binding was the only mechanism for its long duration of action.[80]

The plasmalemma diffusion microkinetic model, outlined above for formoterol,[21] also explains the activity of salmeterol by its partitioning behaviour into the plasmalemma lipid bilayer (Fig. 3). Salmeterol does not appear to cross the membrane to enter the cytoplasm, but rather remains in the outermost monolayer, and slowly diffuses from the membrane ($t_{1/2}$ approx 25 min at 25 °C).[77] This mechanism alone, or together with the 'anchored binding theory', explains the long duration of action of salmeterol. This differs from formoterol, for which long duration of effect is explained by a depot of formoterol molecules in cell membranes in the smooth muscle or in the tissue[72,73] (Fig. 3).

Compared with formoterol, salmeterol is a partial agonist at the β_2-receptor and therefore does not result in maximal bronchodilation.[70,81,82] Salmeterol is appropriate for use as a regular long-acting β_2-agonist, but not for rapid reversion of airflow obstruction or for treatment of acute symptoms of asthma.

Current use of β_2-agonists

Regular use and as-needed use

Since the importance of inflammation in causing the symptoms of asthma was recognised, anti-inflammatory drugs, particularly ICS, have been recommended for first-line maintenance treatment.[83] Bronchodilators, such as the inhaled short-acting β_2-agonists salbutamol (200 or 400 μg) and terbutaline (0.5 mg), are now regarded as symptom-relieving drugs to be given on an 'as-needed' basis.[84] This change in usage, from a regular four-times daily regimen (which, it was thought, would 'keep the airways open') to use only as needed, was catalysed by a study showing that the regular use of the potent short-acting β_2-agonist fenoterol resulted in a significant deterioration of asthma control.[85] This deterioration was accompanied by a decline in lung function and increased airway responsiveness,[86] as well as more exacerbations,[85–87] despite concurrent use of ICS.

The regular use of salbutamol had become widespread as standard practice, based in large part on one very short study.[88] In this crossover study with 1 week of treatment in each arm, slightly higher evening PEF (measured after bronchodilator use) and less need for reliever salbutamol (but overshadowed by eight puffs of regular treatment each day) were regarded as benefits of regular treatment. Extrapolation of regular use of salbutamol to the more potent agent fenoterol seems to have been a major reason for the epidemic of asthma mortality experienced in New Zealand from 1976 onwards. Subsequent work has shown no advantage in patients taking salbutamol on a regular basis over and above its use as needed.[89]

On the other hand, studies with the longer-acting β_2-agonists formoterol (12 μg delivered dose twice daily) and salmeterol (50 μg twice daily) have shown that both improve lung function and increase overall asthma control when used regularly in combination with ICS.[84] In particular, regular use of these agents has been shown to reduce the ICS dose requirement and hence the potential for easy

bruising, skin thinning, cataracts, weight gain and increased incidence of oropharyngeal side effects associated with prolonged high-dose ICS therapy.[90] This is supported in part by a study in which 50% reduction in ICS dose with concomitant salmeterol therapy was well tolerated with no significant loss of asthma control.[91] However, that study also showed that stopping ICS treatment altogether, while continuing salmeterol therapy, resulted in a significant ($P<0.001$) increase in the number of asthma exacerbations. This reinforces the view that although long-acting β_2-agonists have a steroid-sparing effect when used in combination with ICS, they do not show anti-inflammatory activity and when used independently do not maintain as good asthma control.

Combination therapy with salmeterol

Addition of a long-acting β_2-agonist to ICS (combination therapy) has been demonstrated in many studies to be more effective in reducing asthma symptoms and increasing lung function than increasing the ICS dose alone. Studies in patients with asthma who were symptomatic despite maintenance treatment with beclomethasone and who showed bronchodilator responsiveness, have demonstrated that, compared with an increased dose, adding salmeterol to the existing dose of beclomethasone was preferable to increasing the dose of steroid alone. With combination therapy, daytime and night-time symptoms were better controlled, morning and evening PEF rates were improved, and diurnal variation and use of relief bronchodilator medication were reduced.[92,93]

Patients initiated into maintenance treatment with a combination of salmeterol and fluticasone propionate were found to have greater improvements in pulmonary function and symptom control than those initiated on treatment with either therapy alone.[94] Equally, patients symptomatic while receiving low-dose fluticasone propionate (100 μg) achieved significantly greater improvements in lung function and symptom control and fewer exacerbations, when salmeterol was added to their existing dose of steroid, compared with patients whose dose of fluticasone propionate was more than doubled without the addition of salmeterol.[95] However, a study by D'Urzo and colleagues[96] demonstrated no additional benefit of salmeterol on the number of severe exacerbations in patients concomitantly taking optimal anti-inflammatory treatments, although significant improvements in PEF, night-time awakenings and reliever medication use were observed.

The choice of fluticasone dose in such studies is clearly important as a substantial proportion of the therapeutic benefit can be achieved with a total daily dose of 200 μg/day.[97] Although it is reasonable to choose an optimal ICS dose, thus avoiding under treatment, little additional benefit from higher doses would be expected as such doses would be on the plateau of the dose–response curve.

Combination therapy with formoterol

The 12-month FACET (Formoterol And Corticosteroids Establishing Therapy) study was the first investigation into asthma therapy that used reduction in the rates of severe and mild exacerbations as the primary efficacy parameters.[98] The study demonstrated that adding formoterol to two different doses of budesonide reduced rates of severe and mild exacerbations per patient and significantly ($P<0.05$) improved symptom scores, lung function and the need for short-acting β_2-agonists for relief medication compared with increasing the dose of budesonide alone.[98] When formoterol was added to the lower dose of budesonide (100 μg metered dose, twice daily), severe and mild exacerbations were reduced by 26% and 40%, respectively; when the same dose of formoterol was added to the higher dose of budesonide (400 μg metered dose, twice daily), severe and mild exacerbations were reduced by 63% and 62%, respectively. Severe and mild exacerbations were reduced by 49% and 37%, respectively, comparing budesonide 400 μg with 100 μg twice daily without the addition of formoterol. Hence, the FACET study demonstrated reduced exacerbations with a higher dose of ICS, greater than with the addition of a long-acting β_2-agonist, but the greatest benefits were seen when the two were combined.[98]

Adding formoterol to ICS also appears to be at least as effective in improving lung function and controlling asthma symptoms as doubling the dose of ICS. For example, when formoterol twice daily was added to beclomethasone 500 μg daily, the increase in mean morning PEF and decreased use of reliever medication was significantly ($P<0.01$) greater than for patients not taking formoterol whose ICS dose was doubled.[99]

The 12-month OPTIMA (Oxis [formoterol] and Pulmicort [budesonide] Turbuhaler® In the Management of Asthma) study in patients with mild-to-moderate asthma also demonstrated that adding formoterol to maintenance treatment in patients who remained symptomatic despite receiving a low dose of ICS, reduced exacerbations and improved

asthma control more effectively than doubling the ICS dose.[100] However, in steroid-naïve patients, adding formoterol to ICS was no more effective at reducing exacerbations than ICS alone. The slight increase in lung function seen with formoterol represents the bronchodilator response of normal airways and is not of clinical significance.

Current treatment guidelines

The use of combination therapy is now reflected in treatment guidelines. For adults and children over 5 years old with moderate and severe persistent asthma symptoms in whom control is not achieved by low to moderate doses of ICS alone, addition of β_2-agonists with long-acting properties, such as salmeterol or formoterol, to ICS is recommended as preferable to increasing the dose of ICS alone.[4,5,64,82,83]

Safety of β_2-agonists

Side effects of β_2-agonists

Side effects of β_2-agonists are greatest when the drugs are administered orally or parenterally.[84] Unwanted effects include muscle tremor, increases in blood glucose and lactate, and decreases in serum potassium and serum calcium.[26] β_2-agonists can impact cardiac function (producing, for example, palpitations, tachycardia and arrhythmias in rare cases), which must be monitored. Some of these side effects can be considered inevitable considering the pharmacological actions of the drugs and the widespread distribution of β_2-receptors. For example, tremor results from stimulation of the β_2-receptors in skeletal muscle, while even the most selective drugs will have some effect on β_2-receptors in the heart.[56] However, most pharmacologically predictable events appear to be of little clinical significance in patients receiving recommended doses. If the drug is given by inhalation, many side effects are avoided as the systemic load and thus the plasma concentration of the drug is reduced.[56] Patients with more severe airway obstruction may be relatively protected from unwanted side effects due to reduced lung absorption.[101]

Tachyphylaxis to both the bronchoprotective effect, and, to a lesser extent, the bronchodilator activity, occurs with all β_2-agonists[102,103] although this can be attenuated with corticosteroid therapy.[104,105] The significance of such tachyphylaxis has been much debated. Recent concerns have arisen over tolerance to regular long-acting β_2-agonist therapy and whether this reduces the efficacy of rescue β_2-agonists when used for rapid relief of symptoms. While carefully designed studies show reduced effectiveness of short-acting β_2-agonists in increasing lung function or in reversing methacholine-induced bronchoconstriction, studies in the emergency room have failed to show any significant differences in response rates to high-dose short-acting β-agonists as needed for the treatment of acute asthma in patients receiving long-acting β-agonist.[106]

Increased mortality associated with β-agonists

Between 1959 and 1966, the death rate in the UK among patients aged 5–34 years with asthma increased threefold, with similar patterns reported elsewhere.[107] Since asthma had rarely been fatal before and no new environmental factors appeared to be involved, concerns were raised about the use of new medications, in particular pressurised isoprenaline aerosols. These deaths were eventually linked to the use of a particularly strong formulation of isoprenaline (isoprenaline forte: five times stronger than the dose of isoprenaline metered dose inhaler [MDI] used in other countries).[107] The death rate decreased following widespread publicity about possible overuse of this drug and the importance of medical supervision. Isoprenaline was later superseded by more selective β_2-agonists, such as fenoterol and salbutamol. However, fenoterol subsequently became linked to a second epidemic of asthma mortality in New Zealand in the late 1970s.

Fenoterol MDI was introduced into New Zealand in April 1976—the same year that the second epidemic began. Although making up less than 5% of the MDI β_2-agonist market in most countries, fenoterol soon accounted for almost 30% of the total MDI β_2-agonist sales in New Zealand.[108] Fenoterol MDI was dispensed at 200 μg/inhalation (compared with salbutamol at 100 μg/inhalation) and shows similar β_2-receptor selectivity to salbutamol. The somewhat greater magnitude and duration of bronchodilation with fenoterol compared with salbutamol may have led to a delay in patients recognising the severity of an attack and seeking medical help.[109,110] Alternatively, it was suggested that fenoterol was associated with increased mortality by increasing cardiac adverse events.[108] Findings from case–control studies of patients who died from asthma during the late 1970s and 1980s in New Zealand were consistent

with the hypothesis that the use of fenoterol by MDI increased the risk of death in severe asthma.[108,111,112] A clinical study of regular β-agonist therapy with two doses of fenoterol 0.2 mg four times daily, demonstrated a deleterious effect on airway responsiveness, lung function, and clinical control of asthma compared with as-needed β_2-agonist treatment.[85] This supports the hypothesis that fenoterol may have increased asthma severity and fuelled the epidemic of mortality.[113] Time-trend data further supported this hypothesis.[114] Not only did the increased death rate from asthma in New Zealand closely follow the introduction and use of fenoterol and decline after restrictions on fenoterol, but hospital admissions also declined abruptly. This strongly suggests that the adverse effect of fenoterol was on asthma severity and not cardiac toxicity. There was no suggestion of a class effect of inhaled β_2-agonists in the epidemic and no evidence that the increased death rate may have occurred because of underprescribing of ICS, or the influence of social factors, such as unemployment.[115]

Increased mortality has also been associated with long-acting β_2-agonist therapy. In a recent study of salmeterol in patients with asthma, a post-marketing clinical trial was halted early following the discovery of a non-significant trend towards more asthma-related deaths in patients taking salmeterol.[116] African-American patients (17% of study population), especially those not maintained on ICS, had a significantly higher risk of death. These deaths led to a labelling change indicating caution in the use of salmeterol in African American patients.

The interpretation of studies relating to asthma morbidity and mortality, and the associations with potent or frequent use of inhaled short-acting β_2-agonists, has been much debated. Caution was urged over the regular use of all inhaled β_2-agonists based on the study of regular fenoterol vs. as-needed bronchodilator.[85] Sears and Taylor[113] proposed the hypothesis that the increased morbidity and mortality of asthma over the previous decade was related to increased disease severity, as evidenced by increased airway responsiveness related to regular inhaled β_2-agonist use. The abrupt and striking decrease, not only in asthma mortality but also in asthma-related hospital admissions in New Zealand following the withdrawal of fenoterol in 1990, implied a likely causal relationship between fenoterol and asthma severity, rather than cardiac toxicity.[2]

A study commissioned by Boehringer Ingleheim, the manufacturers of fenoterol, of death and near-death events in Saskatchewan, Canada, and the relationship with use of asthma drugs, found an odds ratio for death of 5.4/canister/month with inhaled fenoterol and 2.4/canister/month with salbutamol compared with the reference group of patients with asthma who did not receive any β_2-agonist.[117] Further analysis of this study revealed that the risk of asthma death began to escalate markedly at about 1.4 canisters/month of inhaled β_2-agonist.[118] Svedmyr and Löfdahl[84] argued that, with the exception of nebulised and high-dose fenoterol, no study has convincingly demonstrated a connection between the use of inhaled β_2-agonists at recommended doses and an increase in asthma deaths. A review of the literature on the clinical experience with inhaled β_2-agonists concluded that these agents remain appropriate and reliable treatments for patients with asthma.[119] Nevertheless, the two mortality epidemics linked with high-strength isoprenaline in the 1960s and with fenoterol in the late 1970s, have made most clinicians cautious in prescribing potent short-acting β_2-agonists regularly or frequently as reliever therapy.

Tolerance to β_2-agonists

The development of tolerance to their bronchodilator activity may be a concern with the prolonged use of β_2-agonists due to receptor downregulation and desensitisation. Because of their longer duration of β_2-receptor occupancy, formoterol and salmeterol might be expected to induce even greater tolerance than the short-acting β_2-agonists. However, while a decrease in morning lung function (PEF) has been shown following regular treatment with salbutamol, no tendency to develop tolerance has been shown with salmeterol[120,121] or formoterol.[122–124] Where some tolerance to β_2-agonists has been demonstrated in patients with asthma who experienced severe attacks,[125] this has usually been easy to overcome by increasing the dose slightly.[84]

Patients taking salmeterol both with ICS[92] and without ICS[126] showed no evidence of reduced bronchodilator efficacy over 6 months of study. Equally, the FACET study found no evidence of deterioration in the control of asthma, and although there was an initial decrease in bronchodilator efficacy upon addition of formoterol, the improvements in lung function were maintained at a higher level than with ICS alone for 1 year, showing no further evidence of tolerance (Fig. 4).[98] On the other hand, many studies have found evidence for tolerance to the bronchoprotective effects of long-acting β_2-agonists to allergen or

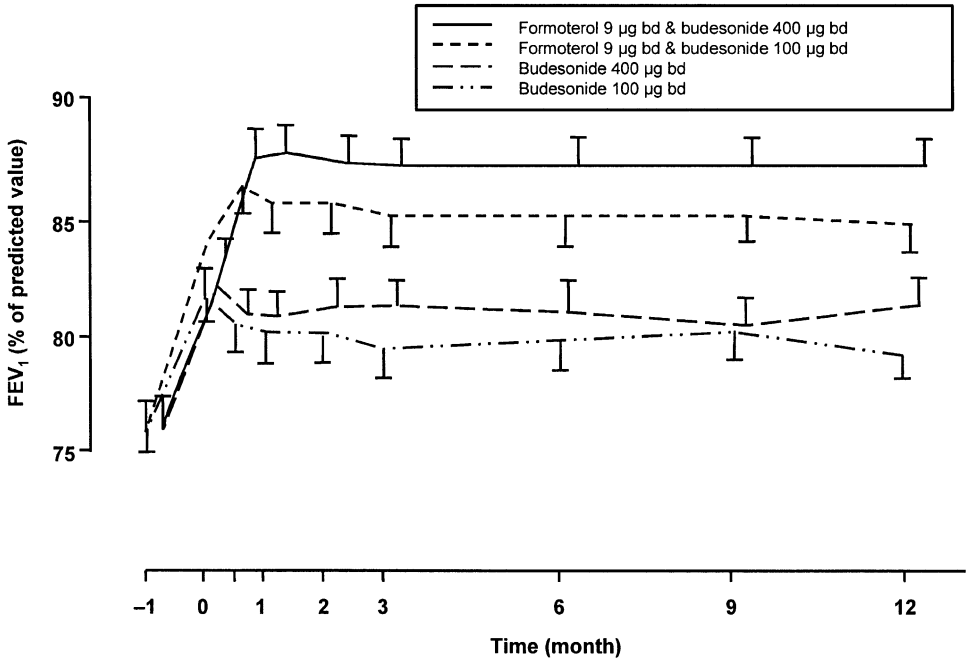


Figure 4 Increase in FEV_1 in patients taking formoterol 9 µg delivered dose twice daily plus budesonide either low dose (100 µg twice daily) or high dose (400 µg twice daily) compared with patients taking the same doses of budesonide alone. Reprinted with permission, copyright© 1997 Massachusetts Medical Society. All rights reserved.[98]

non-specific bronchial challenge. However, as noted by McFadden,[119] even where a significant decrease in protective effect was noted, protection still remained greater for the drugs investigated than with placebo and never fell below pre-treatment baseline values.

Regular use of long-acting β_2-agonists has, however, been linked with tolerance to rapid-acting β_2-agonists. Several studies have reported reduced efficacy of short-acting β_2-agonists following treatment with formoterol or salmeterol.[127–129] The acute administration of a high dose of corticosteroids has, however, been shown to attenuate this effect.[49,51,130] van der Woude and colleagues[128] indicated that both formoterol and salmeterol reduced the effect of salbutamol to a similar extent, whereas van Veen et al.[129] showed higher tolerance with salmeterol and Lee et al.[53] demonstrated higher tolerance with formoterol in similar studies comparing the two long-acting β_2-agonists. In contrast, Nelson et al.[131] and Korosec et al.[106] reported no decrease in efficacy of salbutamol following treatment with salmeterol, demonstrating no clinically important tolerance. The interaction between long- and short-acting β_2-agonists may be associated with prolonged receptor occupancy by long-acting β_2-agonists, preventing binding by other β_2-agonists.[130]

Long-term safety of β_2-agonists

There is growing evidence that β_2-agonists with long-acting properties are safe and effective as an add-on treatment option for patients with asthma poorly controlled by low doses of ICS alone. Improvements in symptoms do not appear to be made at the expense of a worsening of underlying control, and systemic adverse effects resulting from prolonged use of high doses of corticosteroids can be reduced.[132]

Future developments in asthma care

β_2-receptor gene polymorphisms

It is now known that there are subgroups of asthma patients who experience bronchodilator desensitisation related to specific polymorphisms of the β_2-receptor genes.[133] However, while these subgroups of patients may experience reduced benefit from the use of formoterol or salmeterol on a regular basis, there is no evidence that they would not benefit from the rapid onset of formoterol (i.e. if used as needed).[44] Patients with certain β_2-receptor gene polymorphisms have demonstrated lower airway responsiveness to inhaled salbutamol;[40,41] however, reduced bronchoprotection was not evident in similar patients using formoterol.[45]

The presence of at least four polymorphisms of the β_2-receptor gene, discussed earlier, each with different properties and resulting in different responses to β_2-agonist therapy, raises the future possibility of asthma treatments being individually tailored to match patient genotypes.[32] In this respect, it is significant that patients have been identified who did not respond to salmeterol but who still responded to formoterol.[134–136]

β_2-agonists in exercise-induced bronchoconstriction

The long duration of action of formoterol and salmeterol should make these drugs more effective for protection against exercise-induced bronchoconstriction (EIB) than the traditional, short-acting β_2-agonists, such as terbutaline and salbutamol. Salmeterol (50 μg single dose) has been shown to protect against EIB for more than 12 h,[137,138] although the protective effect of salmeterol does decrease when the drug is taken regularly.[139] Similarly, single doses of formoterol delivered by Turbuhaler (6 and 12 μg) gave significantly ($P<0.05$) better and longer protection against EIB in adults[140] and children[141] compared with terbutaline 0.5 mg. Formoterol had about three-times longer duration of effect than the short-acting bronchodilators normally used to protect against EIB.[141]

Regular use of inhaled β_2-agonists can result in a reduction in the protective effect that they offer against bronchoconstrictor stimuli. In one clinical trial, for example, patients with EIB received either formoterol or placebo twice daily for 4 weeks and performed two cycle ergometer tests, 30 min apart, on days 1, 14 and 28; a dose of formoterol was taken 30 min before the second test. Significant tolerance to the protective effect of formoterol was evident in the formoterol patients, but not in the placebo patients, at days 14 and 28 ($P = 0.012$ for both occasions).[142] This is echoed in a meta analysis of corticosteroid-treated patients with asthma.[50] In patients with the Arg16 polymorphism, 1–2 weeks of treatment with formoterol was less effective than placebo. A study of patients with EIB compared the effects of salmeterol with the leukotriene receptor agonist montelukast.[143] This study demonstrated tolerance to the protective effect of salmeterol but not to montelukast over an 8-week period. Tolerance, however, is a class effect that is shared by all β_2-agonists and, although the bronchoprotective effect is slightly reduced with regular dosing, a clinically significant level of bronchoprotection remains in the majority of patients.

β_2-agonists used as needed

Although the β_2-agonists with long-acting properties, formoterol and salmeterol, are playing an increasing role in combination with ICS in maintenance treatment of asthma, a further development is the use of fast-acting formoterol for use as needed for symptom relief.

Formoterol given by Turbuhaler has as rapid an onset of action as salbutamol administered by pressurised MDI (pMDI).[75] Studies have shown that formoterol has a wide safety margin when used as needed in addition to maintenance dosing. Formoterol was well tolerated at daily doses corresponding to 90 μg delivered dose in patients with stable chronic asthma and without concomitant ischaemic heart disease,[144] and in patients with acute bronchoconstriction in an emergency room setting.[145]

A randomised, double-blind, parallel-group, 3-month study compared formoterol 6 μg with terbutaline 0.5 mg (both delivered by Turbuhaler) taken as needed.[124] In this study, patients had moderate-to-severe asthma requiring as-needed medication despite taking an ICS. Patients given formoterol as reliever medication experienced a significantly ($P = 0.013$) longer time to first severe exacerbation (Fig. 5), had greater improvement in lung function and took fewer inhalations of this as-needed medication, compared with patients given terbutaline. Both treatments were well tolerated, with no statistically significant differences between the two treatment groups in electrocardiographic findings, serum potassium concentration or adverse events.

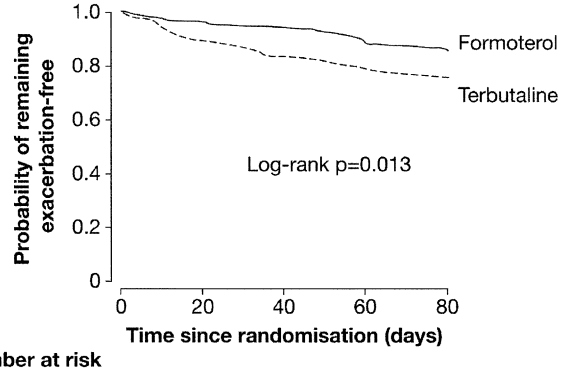


Figure 5 Kaplan–Meier plot showing estimated probability of remaining without severe exacerbation in patients taking formoterol 4.5 μg inhaled dose or inhaled terbutaline 0.5 mg as needed. Reprinted with permission from Elsevier.[123]

Earlier short studies that compared formoterol as needed with salbutamol all demonstrated greater improvement in asthma control with formoterol.[146–148] A recently published, 6-month, real-life study comparing formoterol and salbutamol as reliever medication provided further evidence of the efficacy of formoterol as needed, with a significant ($P<0.001$) reduction in the risk of asthma exacerbations compared with salbutamol.[149] This study was powered primarily for safety outcomes ($n = 18,132$) and confirmed that formoterol used as needed had a safety profile similar to that of salbutamol.

There is thus strong evidence that formoterol's unique combination of long duration and fast onset makes it suitable not only for regular maintenance treatment in asthma (in combination with ICS) but also for use as needed. Formoterol has recently gained FDA approval for use as a rescue medication. This is reflected in the current Global Initiative for Asthma (GINA) guidelines, which has revised its classification of β_2-agonists to rapid-acting and/or long-acting.[64] In the clinical setting, the rapid onset of action of formoterol enabling as-needed use may become equally as important as its property of a long duration of action.

Can β_2-agonists be used as monotherapy?

Neither short-acting nor long-acting β_2-agonists are currently recommended as monotherapy in patients with persistent asthma because of a perceived lack of anti-inflammatory activity.[4,5,64,83] Most studies have found no convincing evidence of reduction in inflammatory cell infiltrates in airway walls or in secretions following use of these agents. Some studies have suggested that treatment with formoterol[150] and with salmeterol[151] can reduce some markers of airway inflammation in patients with asthma and have suggested the intriguing possibility that these drugs may, in fact, be capable of independent anti-inflammatory action. Likewise, recent research has suggested that there may be interactions between long-acting β_2-agonists and corticosteroids at the cellular levels.[152] More research is needed to determine whether this is relevant to the clinical use of these β_2-agonists.

Conclusions

Whether used prophylactically, as needed, or regularly in combination with ICS, β_2-agonists have played a significant role in the treatment of asthma since they were first introduced nearly half a century ago. Although each era has had its drug of choice, the β_2-agonists have shown a continuous evolution, with compounds offering progressively increasing selectivity, combined with faster onset and longer duration of action. This is reflected in the most recent update of the GINA guidelines, which has reclassified the traditional short-and long-acting β_2-agonists into rapid-and/or long-acting β_2-agonists.[64]

Although salbutamol is widely used as reliever medication for acute bronchoconstriction, its short duration of action makes it less appropriate for regular use and unsuitable for relieving night-time symptoms. Drugs with a longer duration of action, such as salmeterol and formoterol, are more appropriate in these circumstances. The combination of the rapid onset and long duration of formoterol provides prompt and long-lasting symptom relief for patients, combined with fewer exacerbations. Further research is needed to assess the benefits that this agent may have in terms of patient quality of life and the economic burden of the disease.

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