Add-on therapy with montelukast or formoterol in patients with the glycine-16 β2-receptor genotype

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Aims We assessed whether montelukast or formoterol provides additive effects to asthmatics not controlled on inhaled corticosteroids, by studying patients who were considered to be genetically susceptible to β2-receptor down regulation and subsensitivity, and who expressed the homozygous glycine-16 β2-receptor genotype.

Methods Fifteen corticosteroid-treated, mild to moderate persistent asthmatics received montelukast 10 mg once daily or formoterol 9 μg twice daily for 2 weeks, separated by a 2-week placebo run-in and washout, in a double-blind, double-dummy, randomized crossover design. Bronchoprotection against adenosine monophosphate (AMP) challenge (primary endpoint), spirometry and blood eosinophils were measured at trough after placebo, first and last doses.

Results For AMP PC20 vs placebo, there were sustained significant (P < 0.05) doubling dilution improvements following first (1.1; 95% CI 0.4, 1.9) and last (1.0; 95% CI 0.3, 1.8) doses of montelukast, and following first (1.3; 95% CI 0.1, 2.6) but not last (0.3; 95% CI −0.9, 1.6) doses of formoterol. Blood eosinophils (×106 l−1) were significantly (P < 0.05) suppressed after the last dose of montelukast (−71; 95% CI −3, −140) compared with placebo, while formoterol exhibited a nonsignificant rise (20; 95% CI −92, 132). Neither treatment significantly improved FEV1, FEF25-75 or PEF after 2 weeks.

Conclusions In genetically susceptible patients with the homozygous glycine-16 genotype, montelukast, but not formoterol, conferred sustained anti-inflammatory properties in addition to inhaled corticosteroid, which were dissociated from changes in lung function after 2 weeks. Thus, assessing lung function may miss potentially beneficial anti-inflammatory effects of montelukast when used as add-on therapy.

Keywords: adenosine monophosphate, asthma, formoterol, genetics, leukotriene antagonists, montelukast, polymorphism, β2-agonists

Introduction

Long-acting β2-agonists such as formoterol and salmeterol are recommended as add-on therapy in asthma when inhaled corticosteroids (ICS) provide suboptimal symptom control [1, 2]. Their addition has been shown to provide equivalent asthma control compared with using a higher dose of ICS alone [3, 4]. However, when given on a regular basis in patients taking ICS, long-acting β2-agonists produce downregulation and desensitization of airway β2-adrenoceptors, resulting in tolerance to their bronchoprotective and bronchodilator effects [3, 5–9].

In vitro studies have demonstrated that susceptibility to β2-agonist-induced downregulation is associated with allelic polymorphisms of the β2-adrenoceptor at positions 16 and 27. At position 16, the homozygous glycine genotype confers increased susceptibility compared with either homozygous arginine or heterozygous genotypes [10, 11]. Polymorphisms at position 16 are also dominant over those at 27 in determining susceptibility. Furthermore, the homozygous glycine-16 genotype has been shown in vivo to predispose to bronchodilator desensitization in asthmatic patients receiving regular inhaled formoterol [12]. Other data have suggested the homozygous glycine-16 genotype to be associated with blunting of the acute bronchodilator reversibility to salbutamol [13,
Patients and methods

Fifteen nonsmoking, mild to moderate persistent asthmatics (5M, 10F; mean ± SEM age 35.1 ± 2.9 years; FEV₁ 79 ± 4.0%; FEF₂₅₋₇₅ 51 ± 4.7%), who had daily symptoms despite being on inhaled corticosteroids (ICS, median dose 500 μg day⁻¹; one on fluticasone, five on budesonide, nine on beclomethasone) and requiring at least two puffs day⁻¹ of rescue bronchodilator, were recruited to completion. All patients exhibited airflow hyper-responsiveness to AMP challenge testing with a provocation concentration producing 20% fall in FEV₁ (PC₂₀) of less than 200 mg ml⁻¹ (geometric mean 18.7 ± 5.7 mg ml⁻¹) prior to the initial run-in period. All patients had the homozygous glycine genotype at position 16. For position 27, two patients were homozygous for glutamine, four were homozygous for glutamic acid, and the remaining nine were heterozygous (i.e. glutamic acid and glutamine). In view of the small numbers of patients with each genotype at position 27, a formal statistical comparison was not made. Genotypes were determined using a previously described method [12].

The study was approved by Tayside Medical Research Ethics Committee. All patients gave written informed consent.

In a randomized placebo-controlled, double-blind, double-dummy, crossover design, patients received 9 μg inhaled formoterol twice daily (b.i.d.) (Oxis Turbuhaler 9 μg actuation⁻¹; AstraZeneca Ltd, Kings Langley, UK) plus placebo tablet once daily, or 10 mg oral montelukast (Singular; Merck, Sharp & Dohme Ltd, Hoddesden, UK) once daily plus placebo Turbuhaler twice daily. Prior to each randomized treatment as a washout period, patients received placebo Turbuhaler and tablet for 2 weeks. Tablets were taken at 08.00 h and Turbuhalers at 08.00 and 20.00 h. ICS dose remained stable throughout the study. First-line rescue bronchodilator was inhaled inhaled ipratropium bromide as required (Atrovent Forte, 40 μg puff⁻¹; Boehringer Ingelheim, Bracknell, UK), with their own β₂-agonist rescue bronchodilator reserved as second-line for use in an acute exacerbation (i.e. for safety purposes). Patients demonstrated optimum use of Turbuhaler at each visit. At the beginning of the trial, a pharmacist sealed the treatments and instruction sheets in envelopes. Compliance was assessed by tick charts and tablet counts. Data with more than 90% overall compliance were considered to be assessable; this was attained in all cases.

Laboratory measurements including AMP, spirometry, exhaled NO measurements and blood eosinophil counts were performed in the morning, after placebo, and first and last doses of randomized treatment. Rescue medication was withheld for 12 h prior to each visit. AMP challenge testing was performed as previously described [30]. If PC₂₀ was not reached after the highest dose of 800 μg ml⁻¹, a censored value of 1600 mg ml⁻¹ was assigned. NO was measured using a LR2000 NO gas analyser (Logan Research, Rochester, UK) as described elsewhere [31] under standardized conditions [32]. Spirometry was performed according to American Thoracic Society criteria [33] using a Vitalograph Compact spirometer (Vitalograph Ltd, Bucks, UK). Eosinophil count was measured using an SE-9000 Haematology analyser (Sysmex UK Ltd, Bucks, UK). Domiciliary peak expiratory flow was measured with a Mini-Wright peak flow meter (Clement Clarke, Essex, UK), with asthma symptoms rated according to a four-point scale: 0 (no symptoms) to 3 (severe symptoms), and rescue bronchodilator requirements were recorded twice daily.
**Statistical analysis**

The study was designed with at least 80% power to detect a 1.0 doubling-dose difference in AMP PC\textsubscript{20} from placebo, with the alpha-error set at 0.05 (two-tailed), and powered to show an add-on effect to ICS (i.e. vs. placebo) but not to compare treatments. The data for AMP PC\textsubscript{20} were log-transformed to normalize the distribution before analysis. Mean of the last 5 days’ domiciliary data of each treatment period were analysed. Comparisons between randomized treatments and placebo were made by analysis of variance followed by multiple range testing with Bonferroni’s correction [set at 95% confidence interval (CI)] to obviate multiple pair-wise comparisons (Statgraphics statistical software package; STSC Software Publishing Group, Rockville, MD, USA).

**Results**

There were no significant carryover effects between placebo values prior to each randomized treatment after run-in and washout for any of the measurements (Table 1). Consequently, a pooled placebo value was used for the purposes of comparing with randomized treatments.

**Surrogate inflammatory markers**

For AMP PC\textsubscript{20} compared with placebo, there were sustained significant ($P < 0.05$) doubling-dilution improvements following the first (1.1; 95% CI 0.4, 1.9) and last doses (1.0; 95% CI 0.3, 1.8) of montelukast (Figure 1, Table 2). Formoterol showed significant improvement after the first (1.3; 95% CI 0.1, 2.6) but not after the last dose (0.3; 95% CI −0.9, 1.6). The individual data for AMP responses showed no differences between the genotypes at position 27, with most individuals exhibiting loss of protection between the first and last doses of formoterol (data not shown). After 2 weeks, montelukast induced a significant ($P < 0.05$) reduction in blood eosinophil ($\times 10^6$ cells$^{-1}$) count after 2 weeks (−71; 95% CI −3, −140) compared with placebo, while there was a non-significant increase after 2 weeks of formoterol (20; 95% CI −92, 132) (Figure 2). There were no significant effects on NO for montelukast or formoterol compared with placebo after 2 weeks (Figure 2).

**Laboratory lung function**

There were significant improvements after the first dose of formoterol compared with placebo for FEF\textsubscript{25-75} and PEF, but not for FEV\textsubscript{1} (Figure 3). No significant differences were found between placebo vs. the last dose of formoterol, or vs. the first or last dose of montelukast for any lung function parameters (Table 2).

**Domiciliary diary cards**

Compared with placebo, both treatments showed significant ($P < 0.05$) improvements in daytime rescue use (i.e. recorded pm) but not for any other endpoints (Table 3).

Table 1 Placebo run-in and washout periods prior to randomized treatments.

<table>
<thead>
<tr>
<th>Run-in placebo</th>
<th>Washout placebo</th>
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<tbody>
<tr>
<td>AMP PC\textsubscript{20} (mg ml$^{-1}$)</td>
<td>34.1 [3.1]</td>
</tr>
<tr>
<td>NO (p.p.b)</td>
<td>11.9 [8.75–15.1]</td>
</tr>
<tr>
<td>EOS ($\times 10^6$ l$^{-1}$)</td>
<td>338 [276–400]</td>
</tr>
<tr>
<td>FEV\textsubscript{1} (l)</td>
<td>2.55 [2.34–2.75]</td>
</tr>
<tr>
<td>FEF\textsubscript{25-75} (l s$^{-1}$)</td>
<td>1.81 [1.59–2.02]</td>
</tr>
<tr>
<td>PEF (l min$^{-1}$)</td>
<td>398 [371–424]</td>
</tr>
<tr>
<td>PEFam (l min$^{-1}$)</td>
<td>385 [370–395]</td>
</tr>
<tr>
<td>RESam (puffs 12 h$^{-1}$)</td>
<td>0.8 [0.7–1.0]</td>
</tr>
<tr>
<td>SYMam (U 12 h$^{-1}$)</td>
<td>0.9 [0.8–1.0]</td>
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</table>

Means [95% confidence intervals] adenosine monophosphate PC\textsubscript{20} (AMP PC\textsubscript{20}), exhaled nitric oxide (NO), blood eosinophil count (EOS), forced expiratory volume in 1 second (FEV\textsubscript{1}), forced mid expiratory flow (FEF\textsubscript{25-75}), laboratory peak expiratory flow (PEF\textsubscript{lab}), domiciliary morning/evening peak expiratory flow (PEFam/pm), daytime/night-time symptom score (SYMam/pm) and daytime/night-time rescue bronchodilator requirement (RESam/pm). There were no significant differences for any endpoints.
Add-on therapy with montelukast or formoterol.

Table 2 Means [95% confidence limits] for laboratory and domiciliary diary card data for pooled placebo and each randomized treatment.

<table>
<thead>
<tr>
<th></th>
<th>Pooled placebo</th>
<th>Montelukast</th>
<th>Formoterol</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>First dose</td>
<td>Last dose</td>
</tr>
<tr>
<td>AMP PC_{20} (mg ml^{-1})</td>
<td>36.3 [27.5–49.0]</td>
<td>79.4* [58.9–104.7]</td>
<td>74.6* [55.9–99.5]</td>
</tr>
<tr>
<td>FEV₁ (l)</td>
<td>2.50 [2.35–2.65]</td>
<td>2.51 [2.38–2.63]</td>
<td>2.47 [2.35–2.60]</td>
</tr>
<tr>
<td>FEF_{25–75} (l s^{-1})</td>
<td>1.85 [1.71–2.00]</td>
<td>1.92 [1.78–2.07]</td>
<td>1.91 [1.76–2.05]</td>
</tr>
</tbody>
</table>

Means [95% confidence intervals] adenosine monophosphate PC_{20} (AMP PC_{20}), exhaled nitric oxide (NO), blood eosinophil count (EOS), forced expiratory volume in 1 second (FEV₁), forced mid expiratory flow (FEF_{25–75}), laboratory peak expiratory flow (PEFlab), domiciliary morning/evening peak expiratory flow (PEFam/pm), daytime/night-time symptom score (SYMam/pm) and daytime/night-time rescue bronchodilator requirement (RESam/pm). *Denotes a significant difference between pooled placebo and randomized treatments.

Figure 2 Change from pooled placebo in FEV₁ and FEF_{25–75}. *P < 0.05 vs. placebo. First dose (□); second dose (■); ns: not significant vs. placebo.

Figure 3 Change from pooled placebo in blood eosinophils and exhaled nitric oxide. *P < 0.05 vs. placebo. (The change in exhaled nitric oxide with montelukast was not significantly different vs. placebo.) First dose (□); second dose (■); ns: not significant vs. placebo.
endogenous catecholamines in their basal state and therefore more prone to subsequent exogenous downregulation. According to the static baseline model, where effects of endogenous catecholamines are less important on pretrial $\beta_2$-receptor regulation, the converse would be expected in terms of exogenous $\beta_2$-agonists promoting further downregulation in the glycine-16 genotype but not in the arginine-16 genotype, in response to exogenous $\beta_2$-agonist therapy. Further large-scale prospective clinical trials with different genotypes at 16 and 27 are required to resolve which baseline model is more important in determining the development of tolerance with long-acting $\beta_2$-agonists.

Previous in vitro and in vivo studies have shown that the susceptibility to tolerance conferred by the glycine-16 genotype overcomes any resistance conferred by the glutamic acid-27 genotype for patients who exhibit this haplotype in combination. However, the situation is potentially even more complex when taking into account all of the different haplotypes with multiple single-nucleotide polymorphisms. We were unable to determine these complex haplotypes because our database is only characterized according to polymorphisms at position 16 and 27.

It was shown in a retrospective analysis by Drysdale et al. [40] that these unique interactions of multiple polymorphisms within a haplotype may determine the acute bronchodilator response to a single dose of salbutamol. Further pharmacogenetic studies are required to prospectively evaluate whether such haplotypes determine the development of tolerance with chronic dosing of long-acting $\beta_2$-agonists for functional antagonism against bronchoconstriction. Indeed, our data revealed marked tolerance following formoterol, which would vindicate the selection of patients with the glycine-16 genotype.

Our sample size precluded formal statistical comparison of the different genotypes at position 27, but we did not observe any obvious trends from inspection of indi-

### Discussion

The results of the present study showed sustained bronchodoprotection against AMP challenge with montelukast but not formoterol in addition to ICS in genetically susceptible patients who would be expected to fare worse with a long-acting $\beta_2$-agonist. This observation is in keeping with studies using indirect challenges in non-genotyped patients, as reported by Wilson et al. [34] with AMP in steroid-treated patients, and by Villaran et al. [35] and Edelman et al. [36] with exercise in mostly steroid-naive patients, where comparisons were made between salmeterol and montelukast. However using the direct stimulus of methacholine challenge in steroid-treated patients with the homozygous glycine-16 genotype, formoterol 9 $\mu$g b.i.d. and zafirlukast 20 mg b.i.d. both exhibited significant residual trough protection after 1 week [37]. The degree of formoterol-induced tolerance for methacholine protection may not be influenced by polymorphisms at position 16 or 27. This is perhaps not surprising given that tolerance to bronchoprotective effects of $\beta_2$-agonists is more pronounced for indirect than direct stimuli [38].

In order to fully understand the pharmacogenetics of $\beta_2$-receptor regulation, it is important to consider the potential role of endogenous catecholamines in the basal state. In vitro data from transfected cell lines has shown that the glycine-16 genotype is more susceptible to $\beta_2$-agonist-induced downregulation than the arginine-16 genotype. According to Liggett, the dynamic baseline model would result in patients with the glycine-16 genotype being susceptible to pretrial downregulation by endogenous catecholamines, such that their receptors would already be downregulated in the basal state, and therefore less prone to further downregulation by subsequent exogenous $\beta_2$-agonist therapy [39]. In contrast, the arginine-16 genotype, according to the dynamic model, would be relatively resistant to the pretrial effects of

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Pooled placebo</th>
<th>Montelukast</th>
<th>Formoterol</th>
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<tbody>
<tr>
<td>PEFpm (l min$^{-1}$)</td>
<td>400 [390–415]</td>
<td>405 [390–415]</td>
<td></td>
</tr>
<tr>
<td>PEFpm (l min$^{-1}$)</td>
<td>420 [400–425]</td>
<td>420 [400–425]</td>
<td></td>
</tr>
<tr>
<td>RESam (puffs 12 h$^{-1}$)</td>
<td>2.4 [1.2–3.6]</td>
<td>2.4 [1.2–3.6]</td>
<td></td>
</tr>
<tr>
<td>RESpm (puffs 12 h$^{-1}$)</td>
<td>2.8* [2.0–3.8]</td>
<td>2.8* [2.0–3.8]</td>
<td></td>
</tr>
<tr>
<td>SYMam (U 12 h$^{-1}$)</td>
<td>0.8 [0.6–1.0]</td>
<td>0.8 [0.6–1.0]</td>
<td></td>
</tr>
<tr>
<td>SYMpm (U 12 h$^{-1}$)</td>
<td>0.7 [0.5–0.9]</td>
<td>0.7 [0.5–0.9]</td>
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</tbody>
</table>

Means [95% confidence intervals] adenosine monophosphate PC$_{20}$ (AMP PC$_{20}$), exhaled nitric oxide (NO), blood eosinophil count (EOS), forced expiratory volume in 1 second (FEV$_1$), forced mid expiratory flow (FEF$_{25-75}$), laboratory peak expiratory flow (PEFlab), domiciliary morning/evening peak expiratory flow (PEFam/pm) and daytime/night-time rescue bronchodilator requirement (RESam/pm).

*Denotes a significant difference between pooled placebo and randomized treatments.
susted after 2 weeks, in keeping with their genetic although a significant increase in FEF 25-75 and PEF was
may occur in combination with any other genotype at position 27.

The degree of tolerance between the first and last dose protection against AMP challenge shown here for formoterol (a 1.0 doubling-dilution trough difference) concurs with previous data reported by Wilson et al. [34] for salmeterol (1.2 doubling-dilution trough difference) and with Aziz et al. [5] for formoterol (1.9 doubling-dilution peak difference) in nongenotyped patients, the latter receiving 18 μg twice daily, as opposed to 9 μg twice daily used here. This in turn would suggest that the bronchoprotective tolerance occurs irrespective of the position-16 genotype for AMP challenge.

The addition of montelukast, but not formoterol, significantly reduced peripheral blood eosinophils but did not significantly reduce exhaled nitric oxide, although the latter showed a trend. A similar additive reduction of blood eosinophils with montelukast has been reported by Wilson et al. and Laviolette et al. [20]. Furthermore, while Wilson et al. showed no additive effects of montelukast on exhaled NO [34], in another study zafirlukast as add-on therapy exhibited a small but significant effect [37]. Montelukast as monotherapy has also been shown to suppress NO [29, 41]. In this respect, it is recognized that low-dose ICS exhibit a maximal suppression of exhaled nitric oxide to near-normal values [25]. In addition, Tamaoki et al. showed that adding pranlukast, compared with placebo, facilitated a halving of the dose of ICS without any increase in levels of NO or serum eosinophilic cationic protein [21].

In terms of laboratory lung function, neither treatment afforded significant improvements in FEV₁ at either dose, although a significant increase in FEF₁₂⁰⁻₁₅₀ and PEF was only shown after first-dose formoterol. For domiciliary measurements, no significant improvement in morning or evening domiciliary PEF or symptoms were found with montelukast or formoterol. Improvements in laboratory and domiciliary pulmonary function have been reported as add-on therapy in comparative studies with salmeterol, formoterol, montelukast or zafirlukast [34, 37, 41–43].

The effects on lung function are always biased towards a bronchodilator such as a long-acting β₂-agonist, especially when patients are selected according to salbutamol reversibility as an inclusion criteria [42–44]. Our patients were not selected on the basis of bronchodilator reversibility. Nonetheless, the significant improvements in PEF and FEF₁₂⁰⁻₁₅₀ after the first dose of formoterol were not sustained after 2 weeks, in keeping with their genetic susceptibility to developing bronchodilator tolerance [12]. However, it is possible that we have missed a small but sustained improvement in lung function, as our study was not powered on this endpoint.

The lack of any additional improvement with montelukast on domiciliary or laboratory PEF in our study is similar to that described by Robinson et al. [45] in more severe patients, although their patients were already on maximal therapy and they did not evaluate any inflammatory endpoints. Our data showing additive significant effects on AMP and eosinophils with montelukast emphasize the point that assessing lung function only will miss potentially beneficial anti-inflammatory effects of leukotriene receptor antagonists.

In summary, we have shown that in patients genetically predisposed to β₂-adenoreceptor desensitization with the glycine-16 genotype, montelukast, but not formoterol, exhibited sustained anti-inflammatory properties additional to inhaled corticosteroid in terms of bronchoprotection and reduction of blood eosinophils, which were dissociated from any changes in lung function after 2 weeks. Montelukast may therefore be a suitable alternative to a long-acting β₂-agonist in patients who are predisposed to tolerance. Moreover, assessing lung function may miss potential anti-inflammatory benefits of montelukast when used as add-on therapy.

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