Comparative trough effects of formoterol and salmeterol on lymphocyte β2-adrenoceptor – regulation and bronchodilatation

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Abstract Objectives: The primary aim of the present study was to evaluate comparative trough effects of formoterol and salmeterol on β2-adrenoceptor regulation and bronchodilator response after regular twice-daily treatment, with a secondary aim to evaluate any possible association with β2-adrenoceptor polymorphism.

Methods: Sixteen asthmatic subjects, with mean (SD) age 33(9) years, all taking inhaled corticosteroids and with a forced expiratory volume in 1 s (FEV1) of 81(12)% predicted were recruited to take part in a randomised single-blind, three-way cross-over study. The subjects received three treatments each for 1 week, with 1-week washout periods in between: (1) formoterol dry powder, 12 µg twice daily; (2) salmeterol dry powder, 50 µg twice daily; or (3) placebo, twice daily. Spirometry and lymphocyte β2-adrenoceptor parameters were measured before the first dose and 12 h after the last dose of each treatment, as well as domiciliary peak flow during each treatment.

Results: There were no differences in β2-adrenoceptor density (Bmax) between the three treatments prior to the first dose; whereas, after the last dose, Bmax was lower with both active treatments than with placebo, but was significant for salmeterol only – a 1.2-fold geometric mean fold difference (95% CI 1- to 1.4-fold), P=0.04. Compared with placebo, there were n=9 of 16 subjects with salmeterol and n=6 of 16 with formoterol who had a greater than 15% fall in Bmax. Post-hoc trend analysis of polymorphism showed that the propensity for downregulation appeared to be related to the occurrence of an allelic substitution of glycine at codon 16 – 8 of 13 for salmeterol versus 5 of 13 for formoterol with a greater than 15% fall compared with placebo. There were no significant differences between salmeterol and formoterol in terms of mean or individual values for downregulation. There was evidence of persistent bronchodilator activity with both active treatments compared with placebo; this was significant for forced expiratory flow rate between 25% and 75% of vital capacity (FEF25–75) – the mean difference versus salmeterol was 0.39 l/s (95% CI 0.06–0.70), P=0.02, and versus formoterol was 0.35 l/s (95% CI 0.16–0.53), P=0.001. These effects were mirrored by significant improvements in morning peak flow rate compared with placebo – mean difference versus salmeterol was 24 l/min (95% CI 7–42), P=0.01, and versus formoterol was 36 l/min (95% CI 25–48), P<0.0001.

Conclusion: There were no differences between regular treatment with formoterol and salmeterol in their effects on lymphocyte β2-adrenoceptor regulation at the end of a 12-h dosing interval, with both drugs exhibiting a residual degree of bronchodilator activity at the same time point. Further studies to evaluate receptor regulation and bronchodilator response are required in susceptible patients who have the homozygous glycine-16 polymorphism.

Key words Downregulation · Bronchodilatation · Polymorphism β2-adrenoceptor

Abbreviations Bmax lymphocyte β2-adrenoceptor binding density; KD lymphocyte β2-adrenoceptor binding affinity as dissociation constant; Emax maximal cyclic-AMP response to isoprenaline; PEF peak expiratory flow; FEV1 forced expiratory volume in 1 s; FEF25–75 forced expiratory flow rate between 25% and 75% of vital capacity; Gly glycine; Arg arginine; Glu glutamic acid; Gln glutamine
**Introduction**

We have previously shown in asthmatic patients that the homozygous glycine polymorphism at codon 16 predisposes to downregulation of lymphocyte β2-adrenoceptors when measured at 36 h after stopping regular treatment with formoterol in a dose of 24 mcg twice daily [1]. A criticism of the study is that, in real-life clinical practice, patients would not usually stop their treatment for 36 h, along with the dose of formoterol being higher than the usual dose of 12 mcg twice daily. We have also found that the homozygous glycine polymorphism at codon 16 of the β2-adrenoceptor is associated with bronchodilator desensitisation [2]. Studies in non-ge-
typed asthmatics with salmeterol and formoterol have shown additive effects to inhaled corticosteroid in terms of parameters of improved asthma control [3, 4, 5].

The primary aim of the present study was to evaluate the comparative effects of two long-acting β2-agonists, formoterol and salmeterol, on β2-adrenoceptor regulation and bronchodilator efficacy at 12 h after regular twice-daily dosing, with a secondary aim to assess any possible association with β2-adrenoceptor polymor-
phism. In order to improve compliance, both drugs were administered by easy-to-use breath-actuated dry-powder inhaler devices. To obviate the criticism of our previous practice, patients would not usually stop their treatment with formoterol in a dose of 24 mcg twice daily. We have also found that the homozygous glycine polymorphism at codon 16 of the β2-adrenoceptor is associated with bronchodilator desensitisation [2]. Studies in non-ge-
typed asthmatics with salmeterol and formoterol have shown additive effects to inhaled corticosteroid in terms of parameters of improved asthma control [3, 4, 5].

The patient’s genotype was not known prior to the study. A post-hoc analysis of their β2-adrenoceptor polymorphism revealed at codon 16 that six subjects were homozygous Gly/Gly, seven subjects were heterozygous Gly/Arg and three subjects were ho-
mozygous Arg/Arg. Whereas, at codon 27, five subjects were ho-
mozygous Glu/Glu, eight subjects were heterozygous Glu/Gln and three subjects were homozygous Gln/Gln. All subjects who were homozygous Glu/Glu were also homozygous Gly/Gly, in keeping with known linkage disequilibrium [9].

**Methods**

**Patients**

Sixteen (ten female, six male) mild to moderate asthmatic subjects with mean (SD) age of 32.9(9.3) years were recruited to take part in the study which was approved by Tayside committee on medical research ethics (Table 1). All the subjects were taking inhaled corticosteroids as a mean (SD) dose of 475(349) mcg/day (5 were taking budesonide and 11 beclomethasone dipropionate). All were using inhaled short-acting β2-agonists (<2 pu/C128s/day) for occasional symptomatic relief, and three subjects were also taking regular treatment with inhaled long-acting β2-agonists (two with formoterol and one with salmeterol). Baseline spirometry at re-
cruitment showed a mean (SD) forced expiratory volume in 1 s (FEV1) of 81(12)% predicted and a forced expiratory flow rate between 25% and 75% of vital capacity (FEF25–75) of 59(16)% predicted. All had asthma, according to American Thoracic Society criteria [8], which was stable for at least 6 months prior to the study. All subjects gave written informed consent prior to entry in the study.

The study had a randomised single-blind (investigator blind) three-
way cross-over design, in which patients received 1 week of placebo, formoterol or salmeterol treatment. There was an initial 1-week run-in period, and there was a minimum 1-week wash-out period between treatments when all β2-agonist therapy was with-
held. From the beginning of the run-in until the end of the study, the patient’s genotype was not known prior to the study. A post-hoc analysis of their β2-adrenoceptor polymorphism revealed at codon 16 that six subjects were homozygous Gly/Gly, seven subjects were heterozygous Gly/Arg and three subjects were ho-
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**Protocol**

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