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Sustained protection against distilled water provocation by a single dose of salmeterol in patients with asthma


ABSTRACT: The long-acting β₂-agonist salmeterol inhibits in vitro the release of inflammatory mediators up to 20 h. These mediators are involved in ultrasonically nebulized distilled water (UNDW)-induced bronchoconstriction. We investigated whether salmeterol provides prolonged protection against UNDW provocation and whether this effect was paralleled by its bronchodilator effects.

Nineteen asthmatic patients (mean forced expiratory volume in one second (FEV₁) 84.8% predicted, mean provocative concentration of histamine producing a 20% decrease in FEV₁ 0.65 mg·mL⁻¹) participated in this randomized, double-blind, placebo-controlled crossover trial. After measuring baseline FEV₁, patients inhaled 50 μg salmeterol or placebo by metered-dose inhaler. FEV₁ was measured after 20 and 40 min, and UNDW provocations and FEV₁ measurements were performed after 10, 20 and 34 h.

Compared to placebo, salmeterol caused marked bronchodilatation from 20 min up to 20 h after inhalation. Salmeterol also provided more than 20 h of protection against UNDW provocation (still more than one doubling dose). Protection beyond the period of bronchodilatation did not occur. Eleven subjects had a significant reduction in provocative dose of UNDW causing a 20% fall in FEV₁ (PD₂₀,UNDW) values between 10 and 20 h, at a time when there was still persistent bronchodilation. No correlation existed between changes in FEV₁ and changes in PD₂₀,UNDW.

From the equations of regression lines between FEV₁ and corresponding PD₂₀,UNDW values it was calculated that only ~25% of the afforded protection was explained by bronchodilatation. In conclusion, a single dose of salmeterol induces both bronchodilatation and protection independently of this bronchodilation against a physiological bronchoconstrictor stimulus for more than 20 h.

The long-acting β₂-agonist salmeterol xinafoate has a higher potency and much longer duration of action than the short-acting β₂-agonists such as salbutamol [1]. Unlike the short-acting β₂-agonists, it has been suggested that salmeterol has some anti-inflammatory properties. In vitro data showed that salmeterol blocked mast cell mediator release 10–35 times more potently than salbutamol, with effects persisting for more than 20 h [2]. Salmeterol, but not salbutamol, also had inhibitory effects on other inflammatory cells such as eosinophils and alveolar macrophages [3], and afforded long-lasting inhibition of increases in vascular permeability [4]. Despite these cellular and vascular effects, evidence that they are of clinical relevance is still lacking. No change in bronchial hyperresponsiveness (BHR) was reported after 6 weeks of treatment with salmeterol [5] and analysis of bronchoalveolar lavage (BAL) cell profile has not shown convincing evidence of an anti-inflammatory effect [6].

On the other hand, Twentymaan et al. [7] suggested that salmeterol has some additional effects, i.e. preventing the increase in BHR after allergen provocation, beyond the time of bronchodilatation. Pedersen et al. [8] also reported that salmeterol blocked the late asthmatic response and increase in BHR after allergen provocation.

In contrast to pharmacological stimuli such as histamine and methacholine, ultrasonically nebulized distilled water (UNDW) induces airway narrowing indirectly, by causing the release of endogenous mediators and possibly by initiating vagal reflex mechanisms [9, 10]. Challenge with UNDW may increase BHR and induce a late asthmatic response, in the same manner as allergen exposure [10]. Thus, the mechanism by which UNDW provocation induces bronchoconstriction is likely to be similar to those involved in asthma provoked by naturally occurring stimuli [9]. If the above-mentioned long-lasting cell-stabilizing effect of salmeterol were present in vivo, this drug might be expected to afford prolonged protection against UNDW provocation.

The present study was, therefore, designed to assess whether a single dose of salmeterol provided long-lasting protection against UNDW provocation and whether or not this was caused by its bronchodilating properties.
Materials and methods

Study design

This randomized, double-blind, placebo-controlled, crossover trial consisted of two identical 3 day study periods, with a minimal interval of 1 week between the start of the two periods, in order to prevent any carry-over effect. Subjects withheld rescue medication (salbutamol 100 μg by metered dose inhaler (MDI)) at least 6 h before each visit and rested for at least 15 min before starting measurements.

On the first day, at 22.00 h, baseline forced expiratory volume in one second (FEV1) was measured. Subsequently, study medication was administered, consisting of two inhalations of 25 μg of salmeterol or placebo by MDI in random order. Flow-volume curves were recorded 20 min after inhalation of the study medication, FEV1 measurements and a UNDW provocation were performed. Baseline FEV1 on the starting evening of both periods had to be within 10%, otherwise the second period was postponed to a later day.

Subjects

Nineteen nonsmoking asthmatic patients (6 males, 13 females) according to the criteria of the American Thoracic Society [11], aged 16–54 (mean 28) yrs, entered the study. Sixteen persons were atopic, defined by an elevated specific immunoglobulin E or positive intracutaneous tests against house dust mite or two of seven other tested common aero-allergens [12]. At study entry, FEV1 had to be ≥50% predicted, and reversibility had to be ≥15% from prebronchodilator values in response to 200 μg salbutamol by MDI. The provocative concentration of histamine causing a 20% fall in FEV1 from post-air volume of inhaled air. After inhalation of 20 L of ambient air through the system, doubling volumes of air with UNDW (3, 5, 10, up to 160 L) were successively inhaled at 5 min intervals. The response to inhaled UNDW was assessed by FEV1 after 90 and 180 s of each dose. The test was stopped if FEV1 dropped by at least 20% or if 160 L of air with UNDW was inhaled. Before and after each test, the nebulizer chamber and aerosol hose were weighted. The cumulative dose of inhaled distilled water in mL H2O causing a 20% fall in FEV1 from post-air values (PD20,UNDW), was calculated by linear interpolation on a semilogarithmic curve.

Pretrial PC20,H was measured according to the method of Cockcroft et al. [13]. In short, the patient inhaled doubling doses of histamine phosphate from 0.03 to 16 mg·mL−1. The test was stopped if FEV1 fell 20% from baseline, and a log dose-response curve was constructed. The PC20,H was calculated in mg·mL−1 by linear interpolation.

Statistical analysis

All PD20,UNDW data were log10 transformed before analysis. FEV1 data were expressed as % pred [15]. To calculate the treatment effect of salmeterol, differences between values (FEV1 and PD20,UNDW) on salmeterol and on placebo were calculated and tested at each time-point with the Wilcoxon signed rank test. The change in UNDW responsiveness (ΔPD20,UNDW) was expressed in doubling doses (DD), calculated as:

\[(\log\text{PD20,UNDW-salmeterol}) - (\log\text{PD20,UNDW-placebo})]/\log2\]

Period and carry-over effects were analysed according to Koch [16]. The coefficient of repeatability for PD20,UNDW was calculated for each subject using the two UNDW provocations in the placebo period (baseline), at the same time of the day (08:00 h) according to the method of Bland and Altman [17]. Correlations between variables were performed with the Spearman correlation test. Regression lines were compared with analysis of variance (ANOVA) of repeated measurements. For multiple comparisons, a Bonferroni correction was used. A p-value of 0.05 or less was considered significant for one test. For multiple comparisons, this boundary was set at 0.01. Data are reported as mean values (SEM).
Results

Patient characteristics are listed in table 1. Seventeen patients completed the study. Two persons (subjects No. 2 and 8) failed to return to the laboratory for lung function and provocation tests for the second treatment period (both after placebo in period one) and were withdrawn from the study. There were no period or carry-over effects between the two study periods at any time-point with regard to FEV1 and PD20, UNDW data.

Table 1. — Characteristics of the study subjects

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Mean 27.6 85.1 22.3 0.65
SEM 2.1 3.7 1.8 0.20

*: reversibility to salbutamol 200 µg by metered-dose inhaler (% change from prebronchodilator value). +: therapy until 6 weeks before participation in the study. PC20,H: provocative concentration of histamine causing a 20% fall in forced expiratory volume in one second; A: anticholinergic; B: β2-agonist; C: cromolyn sodium; IC: inhaled corticosteroids; T: oral theophylline; M: male; F: female.

Table 2. — Individual data of forced expiratory volume in one second (percentage of predicted value)

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Mean 81.7 95.2 80.6 97.5 81.1 94.5 76.4 93.0 82.6 78.7 76.3
SEM 4.4 4.9 4.3 4.8 4.5 5.1 5.1 4.2 5.0 4.7 4.9

SLM: salmeterol.
The early morning dip seen after treatment with placebo (at 08.00 h) (mean change in FEV1 -7.2%, range -28 to +5, compared to 22.00 h) was completely abolished in all but one patient (p<0.001). The next morning (34 h after inhalation), salmeterol no longer provided protection against a morning dip.

**UNDW provocation tests**

Two of the 17 persons differed in their response to UNDW provocation from the others. Subject No. 1 appeared to be unresponsive to UNDW provocation. He recovered very fast from the constrictor effects of UNDW and showed a plateau in reaction of FEV1 at 80% of the post-air values. Subject No. 10 turned out to be refractory to subsequent UNDW provocation tests. PD20,UNDW increased at subsequent tests, and he ended totally unresponsive to the third test (table 3). For these two patients, no real treatment effect of salmeterol could be calculated, but exclusion of their data did not alter the levels of significance for the major outcome variables. The other 15 subjects demonstrated a good short-term reproducibility of PD20,UNDW. The standard deviation of the differences for baseline UNDW provocations was 0.67 DD.

In the whole group (n=17), treatment with salmeterol resulted in protection against UNDW-induced bronchoconstriction for at least 20 h (table 3). Ten hours after the inhalation of salmeterol, a significant increase was observed in the PD20,UNDW of 16.7 (2.3) mL H2O as compared with 3.3 (1.4) mL H2O after placebo (treatment effect of 2.82 (0.35) DD, p<0.0001). In nine of the 17 subjects, the maximum dose of UNDW was reached. In these patients, the total amount of mL H2O inhaled at that time was taken for analysis, since no PD20,UNDW could be reached after salmeterol. Twenty hours after inhalation (at 18.00 h) there was still a significant protection for UNDW, with a PD20,UNDW of 7.0 (1.5) mL H2O after salmeterol as compared with 3.3 (0.7) mL H2O after placebo (treatment effect of 1.09 (0.23) DD, p=0.0008). After 34 h (at 08.00 h) PD20,UNDW values returned to placebo level (4.8 (1.6) mL H2O after salmeterol as compared with 5.0 (2.0) mL H2O after placebo (treatment effect of 0.1 (0.2) DD, p=0.55)).

**Relationship between UNDW provocation and airway calibre**

For each time-point, ΔPD20,UNDW was not correlated with the corresponding change in FEV1 (ΔFEV1) from placebo to salmeterol (all r<0.11, p>0.65).

Figure 1 shows the FEV1 and corresponding PD20,UNDW values of the individual patients 10 and 20 h (fig. 1a), and 20 and 34 h (fig. 1b) after inhalation of salmeterol. From 10 to 20 h, in most individual patients and as a group, FEV1 did not change (p=0.38), while PD20,UNDW dropped significantly (p=0.002, fig. 1b). From 20 to 34 h, both mean FEV1 and mean PD20,UNDW decreased significantly (p<0.001 and p=0.02, respectively), but again some individual patients showed (almost) no decrease in FEV1, while PD20,UNDW dropped (lines appear roughly vertical), while other patients exhibited a decrease in FEV1 with no change in PD20, (lines are more or less horizontal). Both time courses indicate that the protection afforded by salmeterol was independent from bronchodilation.

The slopes of the regression lines through these points after 10 and 20, but not after 34 h on salmeterol and placebo differed significantly from zero (p=0.001 and p=0.03, respectively), indicating a (linear) relationship between starting airway calibre and BHR. The slopes between the regression lines of salmeterol in comparison with placebo were not different at any time-point (all p>0.49), but again, both after 10 and 20 h, the treatment effect of salmeterol was highly significant, placing the lines after salmeterol parallel at a higher level compared to placebo (p=0.000 and p=0.002, respectively). Previously, it has been shown that there is a linear relationship between FEV1 and the provocative concentration of methacholine causing a 20% fall in FEV1 (PC20,M) [18]. Under the assumption of a similar relationship between FEV1 and PD20,UNDW and because the measurements on salmeterol and on placebo are paired, the relationship between PD20,UNDW and FEV1 can be describe statistically with one equation for the regression lines at each time-point:

after 10 h: PD20,UNDW = -0.747 + 1.075 treatment + 0.035×FEV1
after 20 h: PD20,UNDW = -0.636 + 0.408 treatment + 0.028×FEV1

where treatment is assigned a value of +1 for salmeterol and -1 for placebo.

ΔPD20,UNDW is 2.8 and 1.1 DD, and ΔFEV1 is 18.4% and 10.4%, after 10 and 20 h, respectively. It follows from the equation that 10 h after inhaling salmeterol,
the treatment effect is +2.15 DD and the effect through FEV1 is 0.035\times 18.4=0.65, hence 2.8 DD as found in the study. After 20 h, the treatment effect is +0.82 and the effect through FEV1 is 0.028\times 10.4=0.29 (hence the real found protection of 1.1 DD). This means that only 23\% (0.65/2.8\times 100\%) of the afforded protection can be explained by bronchodilation, and 77\% by a direct effect of salmeterol. After 20 h these values are 26 and 74\%, respectively.

**Discussion**

This study shows that a single dose of salmeterol affords both bronchodilation and protection against UNDW provocation up to 20 h in asthmatic patients who did not use anti-inflammatory medication. Protection against UNDW provocation beyond the period of bronchodilation did not occur. In 11 of 17 patients, the inhibition of bronchoconstriction to UNDW decreased significantly between 10 and 20 h, at which time bronchodilation persisted. However, protection was still more than 1 DD after 20 h, and up to this time, only a maximum of 26\% of the protection could be explained by the bronchodilating effect of salmeterol.

The duration of protection against UNDW challenge was in line with the *in vitro* activity of salmeterol. UNDW provocation is thought to be mediated by the release of mast cell mediators [9, 10]. Salmeterol inhibits the release of these mediators from sensitized human lung fragments for more than 20 h [2]. In the present study, a single dose of salmeterol afforded protection at 10 h of almost 3 DD, and, although the magnitude of the protection weaned, protection was still more than 1 DD after 20 h. In this way, salmeterol showed, *in vivo*, a relevant protection during the period of blocking mediators *in vitro* [2].

In accordance with Booth *et al.* [19], no increase in BHR after withdrawal of salmeterol was found in our study. Thirty four hours after inhalation of salmeterol (more than three times the half-life), no rebound BHR to UNDW occurred, the PD20,UNDW being 0.12 DD above placebo.

Salmeterol also induced bronchodilation for more than 20 h, and protected against the early morning dip 10 h after inhalation. Twentyman *et al.* [7] tested bronchodilation of a single dose of salmeterol up to 34 h, but regular measurements were discontinued after 9.5 h. When starting measurements again after 32 h, salmeterol no longer afforded bronchodilation. In a group of asthmatic patients with similar characteristics as in the present study, Rabe *et al.* [20] showed that salmeterol decreased airway tone significantly over a whole 24 h period, compared with placebo. Because of multiple comparisons, however, the bronchodilating effect was not significant beyond 12 h at the individual time-points. Our study clearly shows a bronchodilation up to 20 h, which disappeared after 34 h.

Besides bronchodilation, an important property of salmeterol could be the ability to afford protection of airways smooth muscle against bronchoconstrictor mediators with time-course characteristics different from those observed for bronchodilation [7]. Since baseline airway function correlates somewhat with airway reactivity [18], the inhibitory effect of a bronchodilator could be due to a change in airway calibre. In this study, both bronchodilation and protection lasted more than 20 h but less than 34 h. More measurements of UNDW provocation during this period would be needed to determine exactly the duration of action and to distinguish between protection and bronchodilation. However, we made measurements at 10 h intervals to avoid confounding problems such as a temporary (small) increase in BHR after UNDW provocation [10, 21], and to avoid refractoriness after repeated UNDW measurements [14, 21], which may persist up to 4 h after the last challenge [22, 23]. Despite this, two patients (subjects No. 2 (dropped out) and 10) became refractory to successive UNDW provocations.

No correlation was found between bronchodilation (AFEV1) and protection (APD(20,UNDW)) provided by salmeterol at any time-point, indicating that protection was not caused by bronchodilation. However, the number of patients in our study is probably too small to state that there might not be a correlation with a much larger
population. On the other hand, figure 1 shows that in individual patients the protection afforded by salmeterol is independent of airway calibre, and there were moderate-to-severe responses to inhalation to water at a time when airway calibre was optimal. The regression lines through these points again show a highly significant treatment effect of salmeterol, by shifting the lines at 10 and 20 h parallel to higher levels than after placebo. From the equations of the regression lines on salmeterol and on placebo at the various time-points, it can be calculated that up to 20 h, only a maximum of 26% of the protection can be explained by the bronchodilating effect of salmeterol. Therefore, there seems to be a differential effect of salmeterol on lung function and the response to UNDW. A similar dissociation has been shown with sodium cromoglycate, which had no effect on lung function, but did block UNDW provocation [24]. Conversely, ipratropium bromide in doses up to 160 μg caused bronchodilation, but did not change the response to challenge with UNDW [25].

Other mechanisms are thus likely to be involved in the protective effects of salmeterol against UNDW-induced bronchoconstriction. The term functional antagonism is often used to describe the protective effects of β2-agonists during provocation tests. β2-agonists may prevent smooth muscle contraction, irrespective of the constrictor mediator, by acting on a different receptor on the same cell, which opposes this constriction [26]. In this way, pharmacological effects of β2-agonists are different between smooth muscle relaxation and protection against bronchoconstriction [27]. It has previously been shown that β2-agonists provide true functional antagonistic protection at the level of the smooth muscle against direct pharmacological stimuli as histamine and methacholine [28]. UNDW, however, is thought not to act directly at the level of the smooth muscle, but to induce airway narrowing indirectly [9, 10]. Therefore, mechanisms other than bronchodilation and functional antagonism should be considered to explain this apparent dissociation.

O’Connor et al. [29] showed that β2-agonists have an additional inhibitory nonsmooth muscle effect on bronchoconstrictor stimuli that involve mast cell activation, in affording a greater protection against adenosine monophosphate- than methacholine-induced bronchoconstriction. Salmeterol has several acute anti-inflammatory effects in vitro that may contribute, e.g. the strong inhibition of the release of mast cell mediators [2], involved in the mechanism of action of UNDW. Thus, although the evidence is only indirect, this protection may indicate long-lasting cell-stabilizing effects of salmeterol in vivo up to 20 h rather than functional antagonism.

Finally, in a number of patients, the protection of salmeterol against UNDW decreased, while bronchodilation persisted. This dichotomy between duration of bronchodilation and protection against a bronchoconstrictor stimulus has already been described by Ahrens et al. [30]. These differences in time course could reflect differences in the mechanism for these two β2-agonistic actions. However, an alternative explanation could be the differences in potency of the bronchoconstrictor stimulus. A larger concentration of a β2-agonist may be required to prevent contraction to a potent stimulus as a provocation test, as compared with the concentration of the drug to produce relaxation of the relatively modest level of bronchospasm at baseline [31]. Several studies showed a relationship between bronchodilator dose and the degree of inhibition of provocation [32]. A greater concentration of salmeterol may be required to prevent contraction to UNDW provocation than is required to produce relaxation. However, the concentration of salmeterol required to prevent mast cell mediator release may similarly be higher than the concentration required to prevent contraction of the muscle by the mediators released.

Whether this nonbronchodilator effect of salmeterol also provides clinically relevant effects or persists after prolonged therapy, is at present unclear. No change in BHR was reported after 6 weeks of treatment with salmeterol [7]. On the other hand, salmeterol significantly improved the treatment of (chronic) bronchial asthma and resulted in a clinical significant improvement in quality of life versus placebo and salbutamol [33]. Greening et al. [34] showed that adding salmeterol to inhaled corticosteroid therapy was more appropriate for patients with inadequately controlled asthma on low-dose inhaled corticosteroids than doubling this dose. Finally, in this study, salmeterol afforded a significant protection of more than 20 h against a naturally occurring stimulus, which may be very relevant for asthma management.

In conclusion, our study shows that a single dose of salmeterol in mild-to-moderate asthma causes bronchodilation and protection independently of this bronchodilation against a physiological bronchoconstrictor stimulus for more than 20 h.

References


