Does tachyphylaxis occur to the non-pulmonary effects of salmeterol?

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- 1 The potential for tachyphylaxis to the non-pulmonary effects of salmeterol, a long-acting selective β_2 -adrenoceptor agonist was investigated in 12 healthy male subjects in a double-blind two period crossover study design.
- 2 Subjects received cumulative doses of up to 400 μg (50+50+100+100+100 μg at 45 min intervals) inhaled salmeterol prior to a 13 day dosing schedule of twice-daily inhaled salmeterol 100 μg or placebo. Twelve hours after the last dose of salmeterol or placebo, subjects again received cumulative doses of up to 400 μg inhaled salmeterol.
- 3 Pulse rate, blood pressure, 12-lead ECG, physiological tremor and peak expiratory flow rate (PEFR) were measured before administration of cumulative doses of salmeterol, at 10, 20, 30 and 40 min after each incremental dose of salmeterol and at 4, 6 and 8 h after the first dose. Blood samples were taken for plasma potassium, magnesium, non-esterified fatty acids (NEFA) and blood glucose concentrations at 20 and 40 min after each dose and at 4, 6 and 8 h after the first dose.
- 4 Eleven subjects completed the study. One subject withdrew due to β_2 -adrenoceptor related adverse events. All other adverse events reported were mild in nature.
- 5 Dose-related changes to the effects of salmeterol on pulse rate, QTc interval, tremor, PEFR, blood glucose and plasma potassium were seen, but there was no dose-related effect of salmeterol on blood pressure, plasma magnesium and NEFA.
- 6 Tachyphylaxis occurred to the effects of salmeterol on tremor, QTc and blood glucose. There was no tachyphylaxis to the effects on plasma potassium. Changes in baseline readings after salmeterol made interpretation of the effects seen on pulse rate and PEFR difficult to assess.

Keywords salmeterol metabolic tachyphylaxis ECG β_2 -adrenoceptor agonist

Introduction

Salmeterol (xinafoate) is a selective β_2 -adrenoceptor agonist which produces long-lasting bronchodilation in asthmatic patients [1], and like other inhaled β_2 -adrenoceptor agonists, causes tremor, tachycardia, hypokalaemia and hyperglycaemia at doses greater than those used therapeutically [2, 3].

In both healthy subjects and asthmatics, tachyphylaxis can occur to metabolic, cardiovascular and tremorogenic effects of β_2 -adrenoceptor agonists [4–7] whilst the effects on the lung are variable [8, 9]. However, tachyphylaxis has not been shown to occur to the pulmonary effects of salmeterol after regular treatment in asthmatic subjects [10]. The aim of this study was to investigate in healthy subjects if there was a reduction in the response to increasing doses of inhaled salmeterol following 13 days treatment with twice-daily inhaled doses of salmeterol 100 μg . This would allow us to make comments

on the possibility of down regulation of β_2 -adrenoceptor after regular treatment.

Methods

Subjects

The study was approved by the Independent Glaxo Group Research Ethics Committee. Twelve healthy male subjects, from the Glaxo Group Research volunteer panel aged between 23–45 years and weighing 60.4–95.7 kg took part in a double-blind, randomised 2-way crossover study. Subjects were judged to be healthy and suitable for inclusion following a full medical examination. They gave informed consent in writing before the study

began. Subjects were required to avoid caffeine-containing foods and alcohol for a 24 h period prior to each study day. They were requested not to take any other drug treatment for the entire duration of the study and to avoid strenuous exercise for 12 h before and for the duration of study day.

Methods

Subjects attended the Clinical Pharmacology Unit on the morning of study Day 1, having fasted from midnight. An intravenous cannula (Wallace Y-can) was introduced into a forearm vein for blood sampling. Three resting baseline measurements of pulse rate, blood pressure (Narco Scientific BP203NA), 12-lead ECG (to include corrected QT interval (QTc) (Mac11, Marquette Electronics Inc), physiological tremor and peak expiratory flow rate (PEFR) (Wright's Peak Flow Meter) were measured. Tremor was assessed by attaching a transducer (Endivco 7265-30 piezo resistive accelerometer) to the middle finger of the subject's non-dominant hand. The palm was elevated from the wrist and after a 20 s 'lag' time, tremor was measured for 20 s and the signal analysed using a tremor analyser [11].

Each subject received a cumulative dose of up to 400 µg salmeterol by metered dose inhaler; increments of 50, 50, 100, 100 and 100 µg salmeterol were administered at 45 min intervals. Pulse rate, blood pressure, 12-lead ECG, tremor and PEFR, were measured at 10, 20, 30 and 40 min after each cumulative dose of salmeterol and at 4, 6 and 8 h after the first dose. Dosing ceased if the mean of the four pulse rate measurements taken after each dose increment exceeded 20 beats min⁻¹ compared with pre-treatment values.

Blood samples were taken for plasma potassium, magnesium, non-esterified fatty acids (NEFA) (5 ml, lithium heparin) and blood glucose concentrations (1 ml, fluoride oxalate), before dosing, at 20 and 40 min after each dose and at 4, 6 and 8 h after the first dose. Plasma potassium and blood glucose were measured using a Hitachi 705 Random access analyser, plasma magnesium by colorimetry and NEFA by enzyme assay involving acyl Co A synthetase with u.v. detection. A meal was provided at 4 h after the first dose; glucose and NEFA concentrations were not measured after this time.

On the following morning (Day 2) subjects began a 13-day, twice-daily regimen of 100 µg inhaled salmeterol or placebo. Twelve hours after the last dose (Day 15), subjects returned to the Clinical Pharmacology Unit and again received cumulative doses of up to 400 µg inhaled salmeterol. Measurements were carried out as on Day 1. After an interval of at least 2 weeks the complete study period was repeated for the other treatment.

Statistical analysis was based on parametric methods for each variable apart from the QTc data, where a non-parametric approach was adopted. A log transformation was applied to all the tremor data prior to analysis.

Measurements taken prior to cumulative dosing and during the 45 min time intervals after each dose of salmeterol were summarised by taking the mean values in each of these time intervals. The changes in each parameter after cumulative dosing were summarised by obtaining the slope from a linear regression against

cumulative dose of salmeterol for each subject and each study day separately.

The study was designed to have a power of 80% to detect a change in slope which corresponded to a two and a half-fold increase between treatments in the dose producing an increase of 10 beats min⁻¹ in pulse rate.

Analysis was performed on the response prior to cumulative dosing and on the final response after cumulative dosing as well as on the slope against cumulative dose of salmeterol. The final response after cumulative dosing corresponded to a dose of 400 µg for all the subjects after salmeterol treatment and for eight of the 11 subjects after placebo treatment. For the remaining three subjects after placebo treatment, this represents the response after a cumulative dose of 300 µg salmeterol.

The comparability of the two sequence groups at the start of the second treatment period was assessed by obtaining the difference in responses between the two study days prior to daily treatment. These differences were compared between sequence groups using two-sample *t*-tests [12]. None of these tests proved statistically significant.

The derived responses from the final study day were assessed by analysis of variance allowing for effects due to subjects, periods and treatments. The difference between the responses to cumulative-dose salmeterol following 13 days salmeterol and placebo treatment was calculated in each analysis, together with 95% confidence intervals. Two-sided tests were used to assess the significance of effects.

For the QTc data, corresponding non-parametric methods were used in each case. Values recorded in each time interval were summarised by the median. The changes in each parameter after cumulative dosing were summarised by obtaining a non-parametric slope against cumulative dose of salmeterol [13]. The derived responses were analysed using Wilcoxon tests [14] and corresponding non-parametric estimates of treatment differences and 95% confidence intervals were obtained [15].

Results

One subject was withdrawn from the study on study Day 1, due to β₂-adrenoceptor related adverse effects (headache, tremor, palpitations and nausea), and his results are not included in the analysis. After placebo treatment eight of the 11 subjects received 400 µg total cumulative dose of salmeterol whilst after salmeterol treatment all 11 subjects received 400 µg salmeterol.

All subjects experienced adverse events of a mild nature which included feelings of tremor and increased pulse rate.

Cumulative dose-response curves to salmeterol after regular treatment with salmeterol and placebo are shown graphically in Figure 1 and derived responses before and after regular treatment are shown in Tables 1, 2 and 3.

Mean pulse rate was significantly higher at baseline 12 h after the last dose of regular salmeterol, compared with placebo, by 7 beats min⁻¹. Following both regular salmeterol and placebo, mean pulse rate rose, after salmeterol challenge, to similar values (78 and 82 beats

Figure 1 Cumulative dose-response curve to salmeterol after regular salmeterol 100 µg (■) and placebo (○).

Table 1 Measurements taken prior to cumulative dosing with salmeterol before and following 13 days treatment with placebo or salmeterol 100 µg twice daily

	Pre-treatment		Post-treatment		Post-treatment		
	Placebo Mean	Salmeterol Mean	Placebo Mean	Salmeterol Mean	Placebo – Salmeterol* Mean	95% CI	P value
Pulse rate (beats min ⁻¹)	65	63	63	70	-7	(-12, -2)	0.009
Systolic BP (mm Hg)	115	116	114	115	-1	(-5, 3)	0.686
Diastolic BP (mm Hg)	71	72	71	73	-2	(-6, 2)	0.214
QTc (ms) [†]	405	401	404	404	-2	(-12, 8)	0.662
Tremor (u)*	74	88	98	137	0.71	(0.57, 0.89)	0.007
PEFR (l min ⁻¹)	576	581	573	596	-23	(-45, -1)	0.041
Glucose (mmol l ⁻¹)	4.94	4.90	4.96	4.93	0.03	(-0.14, 0.20)	0.701
Potassium (nmol l ⁻¹)	3.91	3.94	3.92	3.99	-0.07	(-0.28, 0.15)	0.502
Magnesium (mmol l ⁻¹)	0.76	0.76	0.77	0.76	0.00	(-0.03, 0.04)	0.784
NEFA (mmol l ⁻¹)	0.55	0.52	0.62	0.54	0.08	(-0.08, 0.23)	0.293

[†]For QTc, values are medians.

*For tremor, means are geometric and difference is a ratio.

Table 2 Measurements taken after cumulative dosing with salmeterol following 13 days treatment with placebo or salmeterol 100 µg twice daily

	Pre-treatment		Post-treatment		Post-treatment		
	Placebo Mean	Salmeterol Mean	Placebo Mean	Salmeterol Mean	Placebo – Salmeterol* Mean	95% CI	P value
Pulse rate (beats min ⁻¹)	83	82	82	78	4	(-2, 9)	0.165
Systolic BP (mm Hg)	114	113	117	113	3	(-1, 8)	0.151
Diastolic BP (mm Hg)	71	68	69	69	1	(-3, 4)	0.679
QTc (ms) [†]	434	444	436	424	65	(-3, 88)	0.052
Tremor (u)*	229	240	238	193	1.23	(1.02, 1.48)	0.032
PEFR (l min ⁻¹)	603	602	602	597	6	(-11, 23)	0.451
Glucose (mmol l ⁻¹)	6.09	5.86	6.00	5.26	0.74	(0.27, 1.20)	0.006
Potassium (nmol l ⁻¹)	3.32	3.34	3.37	3.49	-0.12	(-0.26, 0.01)	0.066
Magnesium (mmol l ⁻¹)	0.73	0.71	0.75	0.76	0.00	(-0.02, 0.02)	0.867
NEFA (mmol l ⁻¹)	0.74	0.68	0.72	0.66	0.07	(-0.16, 0.29)	0.510

[†]For QTc, values are medians.

*For tremor, means are geometric and difference is a ratio.

Table 3 Slope/100 µg salmeterol before and following 13 days treatment with placebo or salmeterol 100 µg twice daily

	Pre-treatment		Post-treatment		Post-treatment		
	Placebo Mean	Salmeterol Mean	Placebo Mean	Salmeterol Mean	Placebo – Salmeterol* Mean	95% CI	P value
Pulse rate (beats min ⁻¹)	4.9	5.5	4.5	2.0	2.4	(0.8, 4.1)	0.009
Systolic BP (mm Hg)	0.3	-0.6	0.7	0.0	0.7	(-1.1, 2.4)	0.401
Diastolic BP (mm Hg)	0.2	-0.8	-0.3	-0.9	0.6	(-0.5, 1.8)	0.239
QTc (ms) [†]	5.8	10.0	7.7	3.0	5.0	(1.3, 10.3)	0.017
Tremor (u)*	1.33	1.32	1.26	1.11	1.13	(1.07, 1.20)	<0.001
PEFR (l min ⁻¹)	6.8	5.3	7.3	-0.6	7.9	(2.9, 13.0)	0.006
Glucose (mmol l ⁻¹)	0.34	0.32	0.29	0.10	0.19	(0.04, 0.34)	0.017
Potassium (nmol l ⁻¹)	-0.18	-0.20	-0.17	-0.15	-0.02	(-0.08, 0.04)	0.461
Magnesium (mmol l ⁻¹)	-0.01	-0.02	0.00	0.00	0.00	(-0.01, 0.01)	0.946
NEFA (mmol l ⁻¹)	0.05	0.05	0.03	0.03	0.00	(-0.03, 0.03)	0.929

[†]For QTc, values are medians.

*For tremor, slopes are ratios, means are geometric and difference is a ratio.

min⁻¹ respectively), but the increase per 100 µg salmeterol was significantly less following regular salmeterol compared with placebo (2.0 and 4.5 beats min⁻¹/100 µg respectively).

For QTc, baseline values were similar after both regular salmeterol and placebo treatment. After cumulative doses of salmeterol, QTc was significantly lower after the salmeterol treatment (424 ms) than after placebo (436 ms) and the increase per 100 µg salmeterol was significantly less after salmeterol than after placebo (3.0 and 7.7 ms/100 µg, respectively).

Mean tremor measurements were significantly higher at baseline 12 h after the last dose of regular salmeterol, compared with placebo (137 and 98 units respectively). After cumulative doses of salmeterol, mean tremor values were significantly lower after the salmeterol treatment (193 units) than after placebo treatment (238 units) and the slope of the response per 100 µg salmeterol was significantly less following regular salmeterol compared with placebo (11% increase and 26% increase/100 µg, respectively).

Mean PEFR was significantly higher at baseline 12 h after the last dose of regular salmeterol compared with placebo by 23 l min⁻¹. There was no further rise in mean PEFR on salmeterol challenge after regular salmeterol. Mean PEFR rose on salmeterol challenge after placebo to a similar value to that after salmeterol. The slope of the response per 100 µg salmeterol was significantly less following regular salmeterol than placebo (-0.6 and 7.3 l min⁻¹/100 µg, respectively).

Mean blood glucose concentrations were similar at baseline 12 h after the last dose of regular salmeterol and placebo, but after salmeterol challenge the glucose levels were significantly (0.74 mmol l⁻¹) higher after regular placebo treatment compared with salmeterol treatment. The slope of the response per 100 µg salmeterol was significantly less after salmeterol compared with placebo (0.10 and 0.29 mmol l⁻¹/100 µg, respectively).

Mean plasma potassium concentrations were similar at baseline following both regular salmeterol and placebo and following cumulative dosing the fall after salmeterol treatment was 0.12 mmol l⁻¹ less than after placebo treatment. There was no difference in response per 100 µg salmeterol challenge following regular salmeterol or placebo (0.15 and 0.17 mmol l⁻¹, respectively).

No dose-related effects on blood pressure, plasma magnesium and NEFA were measured after regular or cumulative doses of salmeterol.

Discussion

Tachyphylaxis was demonstrated after regular salmeterol to the increases in blood glucose and QTc interval seen after cumulative doses of salmeterol. For both these parameters the baseline values 12 h after both placebo and salmeterol treatments were similar whilst the mean values after cumulative doses of salmeterol together with the slope per 100 µg salmeterol were clearly less after regular treatment with salmeterol.

Tachyphylaxis has been observed previously to the hyperglycaemic response observed after other β₂-adrenoceptor agonists [5, 6, 16] so the results in the present study with salmeterol are consistent with these findings.

It is well documented that β₂-adrenoceptor agonists produce prolongation of QTc at high doses [17, 18]. However, we are not aware that tachyphylaxis to the change in QTc after β₂-adrenoceptor agonists has been observed previously in either healthy or asthmatic subjects. The mechanism for the increase in QTc with β₂-adrenoceptor agonists is not clear. However, both hypokalaemia and β-adrenoceptor stimulation are associated with electrophysiological changes in cardiac muscle [19, 20]. A study which compared the effect of salbutamol and isoprenaline showed that whilst both drugs produced similar effects on QTc, only salbutamol produced effects on plasma potassium [20]. It may be that changes in QTc are a direct cardiac sympathomimetic effect resulting from a local effect on ion exchange across the myocardial membrane. Whatever the mechanism is, it appears from this study that there is a reduction in the prolongation of QTc after regular treatment with salmeterol. As prolonged QTc has been associated in certain circumstances with ventricular arrhythmia [21], this reduction after regular treatment is encouraging.

The interpretation of the effect of regular treatment with salmeterol on the response to pulse rate and tremor is complicated by the change in baseline after salmeterol. Significantly higher values of pulse rate and tremor were

obtained 12 h after the last dose of regular salmeterol compared with placebo. This change in baseline reflects the long duration of action of salmeterol [1, 22], with residual effect from the last dose. Following regular salmeterol and placebo the peak values for pulse rate were similar after a cumulative dose of 400 µg salmeterol; the increase per 100 µg dose of salmeterol was significantly less after salmeterol which may indicate tachyphylaxis. As it is unlikely we were at the top of the dose-response curve for changes in pulse rate and it may be that if we had used higher cumulative doses of salmeterol we would have demonstrated tachyphylaxis more clearly. For tremor despite the change in baseline we were able to show a statistically significant reduction in tremor after 400 µg salmeterol as well as a reduction in the slope of the dose-response curve. Baseline change after long-acting drugs makes tachyphylaxis studies difficult to design. In a previous study we looked for tachyphylaxis to a number of non-pulmonary effects at 12 and 36 h after the last dose of salmeterol. However, despite measuring tachyphylaxis to some of the parameters 12 h after salmeterol the effect had worn off by 36 h [23].

In the present study, unlike previous studies with β_2 -adrenoceptor agonists, tachyphylaxis did not occur to the reduction in plasma potassium although there was a tendency for the falls to be less after regular salmeterol. It is not clear why this was so as it is generally agreed that changes in plasma potassium are mediated through β_2 -adrenoceptors [24]. Large doses of inhaled β_2 -adrenoceptor agonists are well known to cause reductions in plasma potassium [3, 18] and tachyphylaxis has been observed with other β_2 -adrenoceptor agonists [6]. It is possible that tachyphylaxis might have been observed with larger doses or longer treatment with salmeterol or there may have been insufficient power in the study.

In addition all 11 subjects were able to tolerate the 400 µg dose of salmeterol after regular salmeterol whilst only eight of the 11 tolerated the 400 µg dose after placebo

treatment suggesting that tachyphylaxis to the systemic side effects had occurred.

As this study was carried out in a group of healthy subjects it was not anticipated that we would be able to make any statements about tachyphylaxis to the effects of salmeterol on the lung. In these subjects a dose-related increase in PEFR was seen after cumulative doses of salmeterol. In addition, 12 h after salmeterol there was a significant increase in baseline PEFR compared with placebo. Peak PEFR values measured after 400 µg salmeterol were similar after regular salmeterol and placebo although there was a significant difference in slope. As it is likely that subjects were maximally bronchodilated 12 h after the last dose of regular salmeterol, they were incapable of producing a further response.

There were no changes in plasma magnesium concentrations following regular or cumulative salmeterol dosing. There was a small rise in plasma NEFA concentrations following cumulative doses of salmeterol. The latter is likely to have been due to the subjects being fasted during the period of observation and is not considered to be related to salmeterol treatment.

In conclusion tachyphylaxis was observed to the effects of cumulative-dose inhaled salmeterol on blood glucose, QTc and tremor. The results on pulse rate and PEFR were difficult to interpret due to baseline changes. Tachyphylaxis was not observed to the effects on plasma potassium. The cumulative doses of salmeterol used in this study were substantially higher than the therapeutic dose of inhaled salmeterol in clinical practice but nonetheless were reasonably well tolerated. Present results suggest that salmeterol should be well tolerated in patients with continued use. Furthermore, should patients taking regular salmeterol inadvertently receive higher than the recommended dose, it is likely that some of the effects may be less than expected in a naive subject.

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