

Effects of propranolol inhalation on the diurnal increase in FEV₁ and on propranolol airways responsiveness in atopic subjects with asthma

Y Oosterhoff, G H Koëter, D S Postma

Abstract

Background – Propranolol inhalation provocation tests are used to measure indirect airways responsiveness in the investigation of asthma. In this study the effects of repeated propranolol inhalation provocation tests within the same day on normal diurnal variation in the forced expiratory volume in one second (FEV₁) and subsequent propranolol airways responsiveness were investigated.

Methods – Fifteen atopic asthmatic subjects were challenged with doubling concentrations of propranolol at 08.00 and 16.00 hours on the same study day and at 16.00 hours on a control day to exclude changes related to normal diurnal variation.

Results – Mean (SD) baseline FEV₁ at 16.00 hours on the study day was 3.38 (0.23) l, significantly lower than the value at 16.00 hours on the control day of 3.70 (0.24) l ($p=0.001$). No differences were found between the geometric mean provocative concentration of propranolol causing a 20% fall in FEV₁ (PC₂₀) measured on the study day (08.00 hours, 9.3 mg/ml; 16.00 hours, 11.3 mg/ml) and on the control day (16.00 hours 9.3 mg/ml).

Conclusions – The results suggest that propranolol provocation at 08.00 hours has a long lasting effect on FEV₁, thereby counteracting the normal diurnal increase in diameter of the airways. This makes propranolol challenge tests less suitable for studying indirect airways responsiveness in the course of one day. Because the FEV₁ does not return to control values, it is not possible to determine whether tachyphylaxis to repeated propranolol challenge with a time interval of up to eight hours occurs.

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Keywords: asthma, propranolol airways responsiveness, diurnal variation in FEV₁, tachyphylaxis.

Propranolol inhalation provocation tests are used to measure indirect airways responsiveness in the investigation of asthma.¹ Assessment of indirect airways hyperresponsiveness is gaining interest as it may better reflect inflammatory processes in the airways of asthmatic subjects than measurement of direct airways hyperresponsiveness with methacholine or histamine. Enhanced inflammatory activity has been proposed to play a part in the development of nocturnal asthma. Assessment of

indirect hyperresponsiveness within one day might therefore provide more insight into the pathogenetic mechanisms leading to increased nocturnal airways constriction. Little is known about the effects of repeated propranolol inhalation tests within the same day either on baseline forced expiratory volume in one second (FEV₁) or on propranolol airways responsiveness. Measurement of propranolol airways responsiveness appears to be reproducible from day to day when these tests are repeated within a time interval of one week.^{2,3} However, it has been found that the decrease in FEV₁ after propranolol challenge is longlasting, and does not return within 5% of baseline values after 90 minutes.⁴ Furthermore, upon oral treatment with propranolol the inhaled bronchodilator response with isoprenaline is significantly decreased.⁵ As a circadian variation in serum adrenaline levels has been proposed to underlie variations in the diameter of the airways,^{6,7} propranolol challenge tests may interfere with normal diurnal changes in airways diameter. Besides, previous challenge with a non-specific stimulus may either enhance or reduce the sensitivity to the same or another stimulus. Upon oral treatment with propranolol an enhanced responsiveness to histamine has been observed in asthmatic subjects.⁸ On the other hand, a decrease in the airways constrictive response or tachyphylaxis has been reported after repeated inhalation with a variety of airways constrictive stimuli.^{9,10} It has been shown that tachyphylaxis on airways challenge can even last up to six hours. Tachyphylaxis to propranolol inhalation has not previously been investigated.

The aim of this study was to investigate the effects of repeated standardized propranolol inhalation provocation tests within one day on normal diurnal variation in FEV₁ and on subsequent propranolol airways responsiveness in atopic asthmatic subjects. In diurnally active subjects the lowest FEV₁ is reached in the early morning, whereas the highest value is found in the afternoon. Propranolol challenges were therefore performed with an interval of at least eight hours at 08.00 hours and 16.00 hours on the same study day and on a control day at 16.00 hours.

Methods

SUBJECTS

Fifteen atopic asthmatic subjects were recruited from the outpatient clinic of our department of pulmonary diseases and through advertisements in local newspapers. The study had been

Department of
Pulmonology,
University Hospital,
9700RB Groningen,
The Netherlands
Y Oosterhoff
G H Koëter
D S Postma

Reprint requests to:
Professor D S Postma.

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approved by the ethics committee of our hospital. All subjects signed an informed consent agreement. They had a history of episodic dyspnoea or wheezing consistent with the clinical diagnosis of asthma¹¹ and had no concomitant diseases. Further selection criteria were: (1) age between 18 and 45 years; (2) atopy for house dust mite: positive intracutaneous test, expressed as histamine equivalent weal size (HEWS) ≥ 0.9 (Diephuis Laboratories, Groningen, The Netherlands) or raised specific IgE (RAST score $\geq 2 = 0.7$ U/ml; Phadezym RAST, Pharmacia, Uppsala, Sweden); (3) FEV₁ > 1.251 and $> 60\%$ predicted; post-bronchodilator FEV₁ (after salbutamol 200 μg + spacer) $\geq 80\%$ predicted; (4) airways hyperresponsiveness to histamine, defined as the provocative concentration of histamine that caused a 20% fall in FEV₁ (PC₂₀) of ≤ 32 mg/ml (30 seconds inhalation, two minute interval); (5) no use of oral corticosteroids or inhaled corticosteroids in a dose above 800 μg daily during the two months before the study.

STUDY DESIGN

The subjects attended the laboratory on two days. On control day A spirometric measurements and propranolol inhalation provocation tests were performed at 16.00 hours and on study day B at 08.00 and 16.00 hours. These days were planned in a randomized order and separated by 1–5 days. Inhaled bronchodilators were withheld from 24.00 hours on the days before testing.

PROPRANOLOL INHALATION PROVOCATION TESTS

Initial spirometric measurements were performed using a calibrated water-sealed spirometer (Lode BV, Groningen, The Netherlands) according to standardized guidelines.¹² Prechallenge FEV₁ was measured until three reproducible recordings were obtained, and the best of the three was used for analysis. Highest values were used for baseline prechallenge values. Inhalation provocation tests were performed according to a two minute tidal breathing method adapted from Cockcroft and coworkers.¹³ Solutions of propranolol HCl were freshly made every week from powder preparations (Bufa Chemie, Uitgeest, The Neth-

erlands). After inhalation of 0.9% sodium chloride solution, doubling concentrations of propranolol (0.5–32 mg/ml in normal saline) were administered at room temperature as aerosols delivered by a DeVilbiss 646 nebuliser (DeVilbiss Health Care, Somerset, Pennsylvania, USA) connected to the central chamber of an inspiratory and expiratory valve box with an expiratory aerosol filter. Solution output was 0.13 ml/min, while the air pressure control was adjusted to one atmosphere. The challenge was discontinued when FEV₁ had fallen $\geq 20\%$ from the prechallenge level. The PC₂₀ value was calculated by linear interpolation of the last two points of the log concentration-response curve.

DATA ANALYSIS

Values are presented as mean (SD). PC₂₀ values were analysed after base 2 logarithmic transformation. Day to day variability of FEV₁ within each subject was determined as represented by the coefficient of variation (CV% = SD \times 100/mean). After verifying the normal distribution of values, differences in prechallenge FEV₁ and PC₂₀ values at the different days and time points were analysed using the Student's paired, two tailed *t* test. Correlations between variables were performed with the Pearson correlation test. Values of $p \leq 0.05$ were considered statistically significant. All analyses were performed with the SPSS/PC⁺ V4.0 software package (SPSS Inc, Chicago, USA).

Results

SUBJECTS

The characteristics of the subjects and medication use are shown in table 1. Thirteen of the 15 subjects were non-smokers. The mean FEV₁ was 90.3 (3.2)%; airways hyperresponsiveness to histamine ranged from mild to severe (0.11–32.00 mg/ml) with a geometric mean value of 2.2 mg/ml.

PRECHALLENGE FEV₁

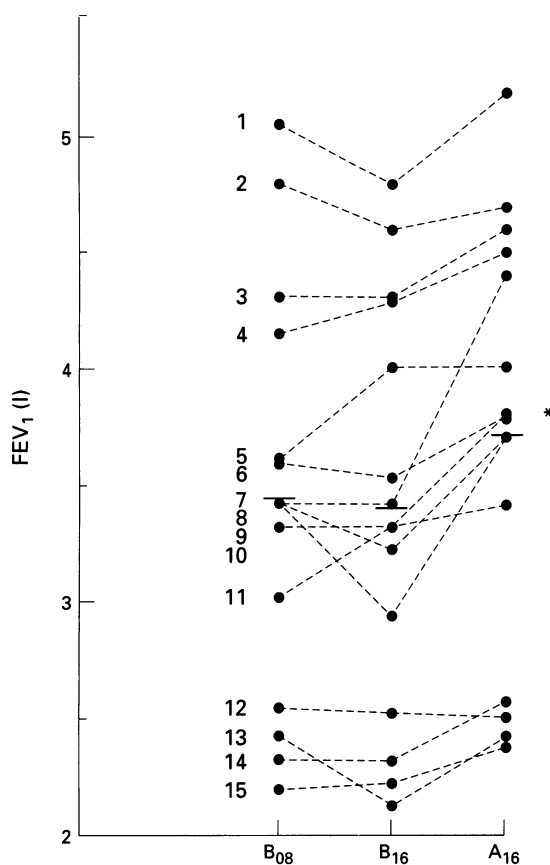
In 13 individuals the FEV₁ values at 16.00 hours on study day B were lower than the values at 16.00 hours on control day A. In subject nos 5 and 12 the FEV₁ values were

Table 1 Details of participating subjects

| Subject no. | Sex | Age (years) | Medication use | HEWS HDM | RAST HDM score | FEV ₁ (% pred)* | PC ₂₀ histamine (mg/ml) |
|-------------|-----|-------------|----------------|----------|----------------|----------------------------|------------------------------------|
| 1 | M | 26 | CS | ND | 2 | 112 | 32.00 |
| 2 | M | 28 | B, A, CS | ND | 4 | 97 | 8.00 |
| 3 | M | 29 | CS | 1.0 | 4 | 107 | 0.36 |
| 4 | M | 32 | — | 1.2 | 2 | 105 | 16.00 |
| 5 | M | 18 | B, Cr | 1.2 | 3 | 86 | 2.88 |
| 6 | F | 21 | B | ND | 4 | 96 | 3.73 |
| 7 | M | 20 | A | 1.2 | 2 | 89 | 4.00 |
| 8 | M | 26 | B, CS | 1.7 | 4 | 86 | 0.12 |
| 9 | F | 22 | B, CS | 1.1 | 3 | 91 | 0.55 |
| 10 | M | 20 | — | ND | 3 | 76 | 1.24 |
| 11 | M | 34 | — | 1.2 | 3 | 75 | 2.54 |
| 12 | F | 40 | B | 0.9 | 0 | 94 | 12.70 |
| 13 | F | 38 | A, Cr, CS | 1.5 | 4 | 87 | 0.11 |
| 14 | M | 36 | B, Cr, CS | 1.6 | 2 | 65 | 2.00 |
| 15 | F | 43 | — | 1.2 | 1 | 89 | 2.73 |

A = anticholinergic; B = β_2 adrenergic; Cr = cromoglycate; CS = corticosteroid inhalations; HEWS = histamine equivalent weal size; HDM = housedust mite.

* Control day at 16.00 hours.



Prechallenge FEV₁ on the study day at 08.00 hours (B₀₈) and at 16.00 hours (B₁₆; eight hours after propranolol challenge) and on the control day at 16.00 hours (A₁₆). Each set of dots represents one individual. The numbers on the left are the subject numbers. Mean values are shown by horizontal bars. * p=0.001 compared with the other time points.

unaltered. No clear difference in subject characteristics or treatment could be detected to distinguish these subjects from the others. The mean FEV₁ at 16.00 hours on day B, eight hours after propranolol provocation the baseline FEV₁ had still not recovered to values comparable with those measured at the control day in 13 of the 15 asthmatic subjects. The mean CV% of FEV₁ at 16.00 hours between the study and control days was significantly higher than that calculated from the normal variability of the day to day measurement of FEV₁ at 16.00 hours in a subgroup of four participating asthmatic subjects. Although we cannot uncritically accept the same day to day variability of FEV₁ for the whole group of 15 subjects, we have no reason to believe that the normal variability at 16.00 hours in the other participating subjects would be much larger. The subjects were all clinically stable and were well trained to perform reproducible spirometric tests. The results from this study therefore suggest that propranolol provocation has a long lasting effect on FEV₁, thereby counteracting the normal diurnal increase in the diameter of the airways.

The mean CV for FEV₁ on the study and control days at 16.00 hours for all 15 subjects was 6.6 (5.4)%. This was significantly different from the calculated reproducibility of the day to day measurement of FEV₁ at 16.00 hours in a subgroup of four subjects (table 2; mean CV 3.4 (1.3)%; p<0.05).

Table 2 Day to day variation of FEV₁ at 16.00 hours

| Subject no. | FEV ₁ | | | | Mean (SD) | CV% |
|-------------|------------------|-----|-----|-----|------------|-----|
| | I | II | III | IV | | |
| 4 | 450 | 440 | 450 | 460 | 450 (8.2) | 1.8 |
| 9 | 380 | 400 | 380 | 410 | 393 (15.0) | 3.8 |
| 12 | 248 | 245 | 265 | 270 | 257 (12.3) | 4.8 |
| 15 | 235 | 240 | 250 | 250 | 244 (7.5) | 3.1 |

Table 3 Airways responsiveness to propranolol (mg/ml)

| Subject no. | Day B | | Day A |
|----------------|-------------|-------------|-------------|
| | 08.00 hours | 16.00 hours | 16.00 hours |
| 1 | 11.3 | 24.8 | 16.8 |
| 2 | 9.0 | 9.0 | 18.0 |
| 3 | 17.0 | 12.0 | 11.0 |
| 4 | 20.8 | 20.8 | 11.7 |
| 5 | 14.0 | 16.0 | 13.0 |
| 6 | 9.2 | 22.2 | 25.6 |
| 7 | 31.0 | 19.0 | 20.0 |
| 8 | 21.1 | 21.2 | 20.0 |
| 9 | 1.4 | 0.8 | 4.1 |
| 10 | 16.0 | 13.0 | 11.0 |
| 11 | 16.0 | 19.0 | 9.0 |
| 12 | 6.0 | 7.7 | 1.2 |
| 13 | 9.0 | 9.0 | 9.0 |
| 14 | 1.0 | 1.0 | 5.0 |
| 15 | 6.6 | 12.4 | 16.0 |
| Geometric mean | 9.3 | 11.3 | 9.3 |

PC₂₀ PROPRANOLOL

All subjects demonstrated airways hyperresponsiveness to propranolol (table 3). Twelve subjects responded to the higher provocation concentrations in the range between 8 and 32 mg/ml. No significant differences were found between geometric mean PC₂₀ values measured at the three time points. Log₂ PC₂₀ propranolol values were significantly correlated with the FEV₁ values on the control day (r=0.56, p<0.05). No relationship was found between log₂ PC₂₀ propranolol values on the control day and log₂ PC₂₀ histamine values (r=-0.03, NS).

Discussion

This study was carried out to investigate the effects of a propranolol inhalation provocation test on baseline FEV₁ and propranolol airways responsiveness in atopic asthmatic subjects within the same day. It shows that eight hours after propranolol provocation the baseline FEV₁ had still not recovered to values comparable with those measured at the control day in 13 of the 15 asthmatic subjects. The mean CV% of FEV₁ at 16.00 hours between the study and control days was significantly higher than that calculated from the normal variability of the day to day measurement of FEV₁ at 16.00 hours in a subgroup of four participating asthmatic subjects. Although we cannot uncritically accept the same day to day variability of FEV₁ for the whole group of 15 subjects, we have no reason to believe that the normal variability at 16.00 hours in the other participating subjects would be much larger. The subjects were all clinically stable and were well trained to perform reproducible spirometric tests. The results from this study therefore suggest that propranolol provocation has a long lasting effect on FEV₁, thereby counteracting the normal diurnal increase in the diameter of the airways.

Furthermore, in this study no effects on propranolol airways responsiveness were found within the same day. PC₂₀ propranolol values were correlated with the FEV₁ values. As the FEV₁ did not return to values comparable with those measured on the control day, the failure to find changes in propranolol responsiveness

could be due to differences in airway calibre. From the results of this study it is not possible to determine whether tachyphylaxis to repeated propranolol challenge with a time interval of up to eight hours occurs.

Propranolol is regarded as a stimulus which indirectly induces airways constriction.¹ It acts by blockade of β adrenergic receptors which are present on many different types of cells located in the walls of the airways, including bronchial smooth muscle cells, cholinergic and non-cholinergic non-adrenergic (NANC) nerves, and mast cells.¹⁴ Beta adrenergic stimulation induces relaxation of airway smooth muscle cells together with inhibition of mast cell mediator release and inhibition of the cholinergic and NANC neurotransmission. Since propranolol induces airways constriction in asthmatic but not in non-asthmatic subjects, it has been proposed that a dysfunction of inhibiting M_2 autoreceptors on cholinergic neurotransmission exists in asthma.¹⁵ As a result of the counteraction of propranolol on the inhibiting β adrenergic stimulation of cholinergic neurotransmission, acetylcholine release increases. In non-asthmatic subjects this leads to an increased negative feedback on cholinergic neurotransmission via M_2 autoreceptors, but in asthmatics the inhibition by M_2 receptors can be impaired as a result of interference by inflammatory mediators. Evidence for an indirect action on the cholinergic neurotransmission is supported by the protective effect of atropine and oxitropium bromide on propranolol induced airways obstruction.^{3,16} Involvement of NANC neurotransmission is supported by the finding that inhalation of vasointestinal peptide decreases propranolol airways responsiveness.¹⁷ Finally, pretreatment with disodium cromoglycate protects against propranolol induced airways obstruction, suggesting involvement of mast cell release or axon reflex mechanisms.¹⁸

In conclusion, this study demonstrates that propranolol inhalation opposes the normal diurnal increase in FEV₁ in most asthmatic subjects. When assessing indirect airways responsiveness within the same day it must be taken into account that the effect of propranolol inhalation on FEV₁ lasts up to eight hours and makes propranolol challenge tests less suitable for studying indirect airways responsiveness within one day. Other indirectly acting stimuli

are probably more convenient. Results from this study also indicate that it is not possible to determine whether tachyphylaxis occurs following repeated propranolol challenge with a time interval up to eight hours.

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