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Protective effect of bronchial challenge with hypertonic saline on nocturnal asthma

M.C. Borges, E. Ferraz, J. Terra-Filho and E.O. Vianna

Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil

Correspondence to: E.O. Vianna, Divisão de Pneumologia, Departamento de Clínica Médica, FMRP, USP, Av. Bandeirantes, 3900, 14048-900 Ribeirão Preto, SP, Brasil
Fax: +55-16-3633-6695. E-mail: evianna@uol.com.br

Inhalation of hypertonic saline (HS) causes bronchoconstriction in asthmatic subjects. Repeated inhalation of HS leads to substantially reduced bronchoconstriction, known as the refractory period. Refractoriness due to different stimuli has also been described (cross-refractoriness). Nocturnal asthma is defined as an increase in symptoms, need for medication, airway responsiveness, and/or worsening of lung function that usually occurs from 4 to 6 am. Our objective was to determine the effect of refractoriness on nocturnal asthma. The challenge test consisted of inhalations of 4.5% saline with increasing durations until a reduction of 20% in forced expiratory volume in 1 s (FEV_1) ($PD_{20}HS$) or total time of 15.5 min. Twelve subjects with nocturnal asthma were challenged with HS at 16:00 and 18:00 h and FEV_1 was measured at 4:00 h. One to 2 weeks later, FEV_1 was determined at 16:00 and 4:00 h. $LogPD_{20}HS$ at 18:00 h was significantly greater than $logPD_{20}HS$ at 16:00 h, 0.51 ± 0.50 and 0.69 ± 0.60 mg, respectively (P = 0.0033). When subjects underwent two HS challenges in the afternoon, mean (± SD) FEV_1 reduction was 206 ± 414 mL or 9.81 ± 17.42%. On the control day (without challenge in the afternoon) FEV_1 reduction was 523 ± 308 mL or 22.75 ± 15.40% (P = 0.021). Baseline FEV_1 values did not differ significantly between the control and study days, 2.48 ± 0.62 and 2.36 ± 0.46 L, respectively. The refractory period following HS challenges reduces the nocturnal worsening of asthma. This new concept may provide beneficial applications to asthmatic patients.

Key words: Refractory period; Bronchial challenge; Hypertonic saline; Bronchial provocation test

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Introduction

Inhalation of hypertonic saline (HS) causes bronchoconstriction in asthmatic subjects (1). Neurogenic reflexes, mediators of inflammatory cells and epithelial cells in the airway mucosa are thought to be involved in this event (2). Over the past 20 years, bronchial challenges with HS have been increasingly used as a relevant test for the diagnosis, assessment of severity and evaluation of treatment of asthma (3). The HS challenge has been shown to be simple, safe and reproducible (4).

After HS-induced bronchoconstriction, asthmatic subjects develop a refractory period during which a second identical challenge induces a substantially reduced bronchoconstriction (5). The refractory period is not a specific phenomenon for HS and its duration and intensity depend on the bronchoconstrictor stimulus (6-9). Refractoriness has been mainly studied as a confounding factor when tests are performed in close sequence and may be used to clarify the pathophysiology of bronchoconstriction, although the mechanisms involved are not understood.

Several studies have demonstrated the presence of cross-refractoriness, a phenomenon whereby a bronchial challenge with one stimulus inhibits the bronchoconstriction induced by another stimulus (10-12). However, no study has investigated cross-refractoriness between a bronchial challenge and the overnight worsening of asthma, a naturally induced bronchoconstriction.

Nocturnal asthma is a common and potentially fatal complication of asthma, defined as an overnight decrease in

lung function that occurs in association with increased airway hyperresponsiveness (13,14) and airway inflammation (15) compared the awake baseline and leads to nocturnal symptoms such as cough and dyspnea (16). The importance and singularity of nocturnal asthma have led to a search for specific medications and dosing schedules (17,18).

We hypothesized that refractoriness induced in the evening could attenuate the nocturnal worsening of lung function in subjects with nocturnal asthma. Therefore, we determined the effect of HS challenges on the overnight reduction in forced expiratory volume in 1 s (FEV_1) of nocturnal asthma.

Subjects and Methods

Subjects

Before entry into the study, all subjects were screened by medical history, physical examination, routine laboratory tests, and 1-week peak expiratory flow (PEF) measurements. Twelve subjects participated in the study. All subjects had asthma as defined by the Global Initiative for Asthma (19). They were non-smokers and had an FEV_1 greater than 50% of their predicted values. None was using theophylline, anticholinergic drugs or had required oral corticosteroids for at least 1 month before entry into the study. Subjects with an upper respiratory tract infection or asthma exacerbation in the last 4 weeks were excluded. All subjects had nocturnal asthma defined as the presence of nocturnal symptoms, with nocturnal awakening and necessity of a bronchodilator, associated with a fall in PEF of >15% from bedtime to morning on at least 4 nights over a 7-day period of testing or a mean PEF fall of >15% from bedtime to morning over a 7-day period. Patients characteristics are given in Table 1. The study was approved by the local Ethics Committee and all subjects gave written informed consent to participate.

Table 1. Subject characteristics and medications.

Subjects	
No. of subjects	12
Gender (male/female)	4/8
Age (years)	33 ± 8
FEV_1 (L)	2.48 ± 0.62
FEV_1 (%)	74.42 ± 13.92
Medication in use	
ß-agonist (short-acting)	9/12
ß-agonist (long-acting)	4/12
Inhaled corticosteroids	9/12
Theophylline	0
Ipratropium bromide	0

Age and lung function parameters are reported as mean ± SD. FEV_1 = forced expiratory volume in 1 s.

Study design

Subjects attended the laboratory on 2 different days, which were called study and control days. The order of these days was randomized and with an interval of 1-2 weeks. On the study day, the asthmatics were submitted to HS challenge at 16:00 and at 18:00 h. No medications were given after each challenge, and the spontaneous recovery of FEV_1 was measured. During the same night, at 4:00 h, the FEV_1 was assessed by spirometry to calculate the nocturnal reduction of FEV_1. On the control day, FEV_1 was measured at 16:00 h and inhalation of isotonic saline was performed after this spirometry (sham challenge). During the same night, FEV_1 was assessed at 4:00 h to calculate the nocturnal reduction of FEV_1. The control day was used to determine the basal overnight reduction in FEV_1. No medications were taken between 16:00 and 4:00 h. After each test, subjects were asked to record their respiratory symptoms. Inhaled short-acting ß-agonists were stopped for at least 12 h and long-acting ß-agonists were stopped for 24 h before the study procedures. The use of inhaled corticosteroids was not stopped.

Hypertonic saline challenge

The HS challenge was performed using an ultrasonic nebulizer (DeVilbiss Ultra-Neb 2000, DeVilbiss, Somerset, PA, USA) calibrated to produce an aerosol output of at least 1.5 mL/min. HS (4.5% NaCl, w/v) was prepared by dilution of commercial sterile preservative-free 20% saline. The aerosol was delivered to the patient by a two-way non-rebreathing valve (Hans Rudolph 2700 series, Kansas City, MO, USA) through 100-cm long tubing with an internal diameter of 2.2 cm. Previous studies have shown that the output of this nebulizer system ranges from 1.9 to 2.5 mL/min at tidal volumes of 300-500 mL and respiratory rates of 12-20/min, with a particle size distribution of median mass aerodynamic diameter between 2.33 and 2.87 µm and with 100% of the particles being less than 5 µm in diameter (20).

After three reproducible measurements of baseline FEV_1, subjects breathed through the valve with the nebulizer switched off for 2 min, FEV_1 measurement was repeated, and then HS inhalation was initiated with a first exposure period of 30 s. After this first inhalation, subjects breathed increasing doses of HS by doubling the duration of nebulization (0.5, 1, 2, 4, and 8 min). FEV_1 was measured in duplicate 90 s after each inhalation. If the FEV_1 reduced less than 10% of the baseline value, the exposure time was doubled. If the reduction of FEV_1 was more than 10% and less than 20%, the exposure time was repeated

rather than doubled. The test was stopped when a reduction of 20% or more of FEV_1 was obtained or after a total exposure of 15.5 min. The aerosol output was determined by weighing the canister and tubing on an electronic scale (AS 2000, Marte, São Paulo, SP, Brazil) before and after the challenge tests. Patients were asked about the intensity of dyspnea at each inhalation by using a scale from 0 to 3, where 0 meant no dyspnea; 1, mild dyspnea; 2, moderate dyspnea, and 3, severe dyspnea. The tests were performed with a Koko spirometer and software (PDS Instrumentation, Inc., Louisville, CO, USA).

Expression of airway responsiveness and refractory period

We plotted FEV_1 results (ordinate) against HS dose (abscissa) in order to construct a dose-response curve. The dose required to produce a 20% reduction in FEV_1 ($PD_{20}HS$) was calculated by linear interpolation between the last two values.

The refractory period was determined by comparing the $PD_{20}HS$ at 16:00 h with the $PD_{20}HS$ at 18:00 h on the study day. To quantify the HS refractoriness, a refractory index was calculated by dividing the 16:00-h $PD_{20}HS$ by the 18:00-h $PD_{20}HS$ (21).

Effect of refractoriness on the overnight FEV_1 reduction

The effect of refractoriness on the overnight FEV_1 reduction was determined by comparing the FEV_1 reduction from 16:00 to 4:00 h on the control day (basal reduction) with the FEV_1 reduction from 16:00 to 4:00 h on the study day.

Spontaneous recovery of FEV_1

The recovery time (spontaneous recovery of FEV_1) was obtained by measuring FEV_1 every 5 min after the challenge during the first hour and then at 15-min intervals until FEV_1 spontaneously returned to 90% of the basal value.

Statistical analysis

The baseline FEV_1 measurements before the bronchial challenges and at 16:00 h on the study and control days were compared by the Student *t*-test. $PD_{20}HS$ values were log transformed and compared by the two-tailed paired Student *t*-test. The overnight reduction in FEV_1 and the recovery time were also compared by the two-tailed paired Student *t*-test.

Results

Repeated challenge of the airways with HS resulted in loss of airway responsiveness. The $logPD_{20}HS$ at 18:00 h (0.69 ± 0.60 mg) was significantly greater than the $logPD_{20}HS$ at 16:00 h (0.51 ± 0.50 mg; P = 0.0033). The mean refractory index was 1.66 ± 0.57 and ranged from 0.68 to 2.35. Baseline FEV_1 values did not differ significantly between the 16:00- and 18:00-h bronchial challenge, 2.36 ± 0.46 and 2.31 ± 0.49 L, respectively.

The overnight reduction of FEV_1 was significantly greater on the control day than on the study day (Figures 1 and 2).

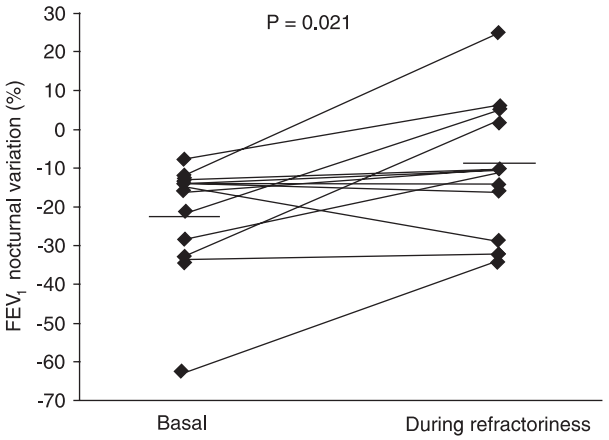

Figure 1. Overnight forced expiratory volume in 1 s (FEV_1) variation on the control and study days. FEV_1 nocturnal variation (reported as percent of basal value) of 12 asthmatic subjects on the control day (basal FEV_1 overnight reduction) and study day (following challenges). On the study day, subjects underwent hypertonic saline challenges at 16:00 and 18:00 h. The horizontal lines indicate the means.

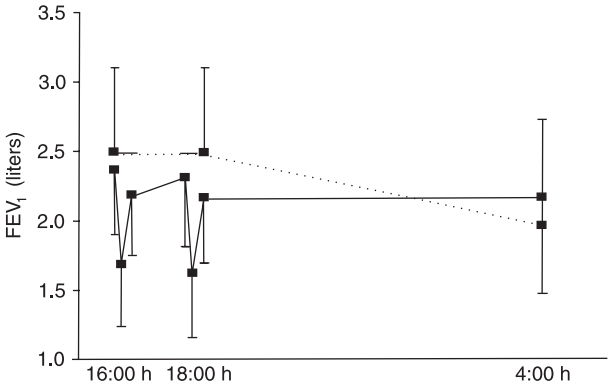

Figure 2. Variation of forced expiratory volume in 1 s (FEV_1) on the control and study days. FEV_1 is reported in liters from 16:00 to 4:00 h for 12 asthmatic subjects on 2 days. On the control day (dotted line) the asthmatics inhaled isotonic saline and on the study day (solid line) they underwent hypertonic saline challenges at 16:00 and 18:00 h. No medications were taken between 16:00 and 4:00 h.

The mean overnight reduction of FEV_1 was 523 ± 308 mL or 22.75 ± 15.40% on the control day and 206 ± 414 mL or 9.81 ± 17.42% on the study day, i.e., during the refractory period (P = 0.021). Baseline FEV_1 values did not differ significantly between the control and study days, 2.48 ± 0.62 and 2.36 ± 0.46 L, respectively.

The mean recovery time after the 16:00-h HS challenge was 37.50 ± 21.58 min and ranged from 10 to 70 min. The mean recovery time after the 18:00-h HS challenge was 31.67 ± 27.83 min and ranged from 5 to 90 min. The recovery time after the 16:00-h HS challenge did not differ significantly from the recovery time after the 18:00-h HS challenge. After the spontaneous recovery, FEV_1 returned to 2.18 ± 0.43 and 2.16 ± 0.46 L, following 16:00 and 18:00 h challenges, respectively (Figure 2).

All subjects reported 0 (zero) for dyspnea before the challenge. The highest degree (score 3) was observed in two (17%) subjects, occurring after the final inhalation. The HS inhalation and the recovery time were well tolerated, with very few side effects.

Discussion

This study demonstrated that performing HS challenges in the evening considerably reduces the overnight FEV_1 fall in subjects with nocturnal asthma. Since no medications were taken between 16:00 and 4:00 h, the improvement in nocturnal bronchoconstriction could not be due to additional use of rescue medication. The protection may be explained by refractoriness. The rationale for this explanation is the cross-refractoriness that has been described among the various bronchoconstrictor stimuli (5,10-12). The mechanisms of nocturnal asthma are complex and have only been partially elucidated (22). Thus, some pathway that is able to induce a bronchospasm and refractory period could be shared between nocturnal and induced bronchospasms.

To provide mechanistic insight for the observations presented, a different group of 13 asthmatics in a controlled and randomized protocol underwent HS challenge at 7:00 and 17:00 h and, on another day, at 17:00 h (control). The PD_{20} at 17:00 h on the study day was significantly greater than the PD_{20} at 17:00 h on the control day (Borges MC, Ferraz E, Terra-Filho J, Vianna EO, unpublished results). Therefore, the duration of the refractory period following hypertonic saline challenges was at least 10 h, which is compatible with the protection observed in the present study.

Some other studies have evaluated the duration of the refractory period. For methacholine, refractoriness has been demonstrated to last 24 h in non-asthmatic subjects (23) and in asthmatics subjects (6). Schmidt et al. (7) have shown 3-day tachyphylaxis after bradykinin challenges. Previous studies on HS challenges demonstrated the occurrence of refractoriness, but not its duration. Further studies are necessary to determine the entire duration of this phenomenon after an HS challenge.

Non-isotonic solutions cause bronchoconstriction in asthmatic subjects by several mechanisms: change in airway osmolarity or ion concentration, mast cell degranulation, airway epithelial alteration, airway smooth muscle contraction, and inflammatory cell and sensory nerve activation (2,24). Although the present study reports for the first time the cross-refractoriness between a bronchial saline challenge and the overnight worsening of asthma, the precise explanation for this phenomenon will depend on a multi-factorial approach, probably with animal models. The pathophysiology of the refractory period has been investigated for approximately 30 years and has not been fully elucidated. Several mechanisms have been evaluated, with no evidence of mast cell mediator depletion (21), catecholamine production (25) or a direct action on smooth muscle (26).

The production of inhibitory prostanoids and neural mechanisms is a possible mechanism. Some studies have demonstrated that the release of inhibitory prostaglandins, probably PGE_2, could have an important role in refractoriness since pre-treatment with indomethacin reduced the refractory period in response to some bronchoconstrictor stimuli (27,28). Other studies have demonstrated that PGE_2 has a bronchodilator and anti-inflammatory effect and reduces airway responsiveness (29).

The contribution of neural mechanisms to the refractory period has been shown in some studies. Rajakulasingam et al. (11), in 1995, demonstrated cross-refractoriness between HS and bradykinin challenges and speculated that these two agonists produced refractory periods through similar pathways. Since neural mechanisms have an important role in bradykinin-induced bronchoconstriction, they probably also contributed to the refractory period (11).

In addition to the pathophysiological relevance, the implications of these findings are quite extensive. These results create a need for further investigations in order to understand the clinical relevance of the phenomenon demonstrated. HS has some benefits in the treatment of allergic rhinitis and cystic fibrosis (30,31) and a salt chamber has been shown to be beneficial to asthmatic patients (32). Since bronchial challenge with exercise has some similarity to HS challenge, we may also speculate on the effect of exercise bronchoconstriction on nocturnal asthma. Nevertheless, in view of the refractoriness following exercise-

induced bronchospasm, it has been reported that exercise is useful for asthmatics (33).

The recovery of bronchospasm and the risk of late asthmatic reaction are issues related to the safety of employing challenge tests. We have shown here rapid, spontaneous and well-tolerated recovery with no need for a bronchodilator. Moreover, up to 10 h after the HS challenge there was no evidence of a late asthmatic reaction.

HS challenges applied in the evening attenuated the overnight reduction of FEV_1 in patients with nocturnal asthma. This observation shows for the first time a new and interesting concept. This protection may be attributed to the release/production of inhibitory prostanoids, to neural mechanisms, reduction of airway responsiveness and/or action on neurogenic inflammation. Further studies are necessary to elucidate these mechanisms and the clinical implications of refractoriness induced by HS in asthma.

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