

Response to inhaled albuterol during nocturnal asthma

Leslie Hendeles, PharmD,^a Russell Beaty, MD,^d Richard Ahrens, MD,^e
Gary Stevens, PhD,^b and Eloise M. Harman, MD^c Gainesville, Fla,
Birmingham, Ala, and Iowa City, Iowa

Background: During conventional daytime studies of β_2 -agonists, 1 puff of a metered-dose inhaler often produces a near maximum bronchodilator response. Consequently, the US Food and Drug Administration–approved dose of albuterol is only 1 to 2 puffs every 4 to 6 hours.

Objective: To determine whether a higher dose of albuterol is required to normalize lung function during nocturnal asthma. **Methods:** Fifteen subjects (age, 18–37 years) were treated with albuterol metered-dose inhalers in a randomized crossover manner at the onset of nocturnal symptoms while sleeping in the Clinical Research Center and during the day when they were asymptomatic. The dose was doubled at 15-minute intervals to 16 cumulative puffs.

Results: The mean $\pm$ SD predose FEV₁ was lower at night than during the day (44% $\pm$ 12% vs 68% $\pm$ 9% predicted; $P = .0001$). The maximum FEV₁ achieved was also lower at night (84% $\pm$ 15% vs 90% $\pm$ 12%; $P = .02$). The nocturnal dose-response curve was shifted to the right. The median (25th, 75th percentiles) dose required to achieve 80% of the subject's personal best FEV₁ was substantially higher at night (5 [1, 19] vs 0.4 [<0.25 , 2] puffs; $P = .02$), and the median time to achieve this endpoint was longer (47 [21, 90] vs 10 [0.2, 42] minutes; $P = .005$). No significant systemic effects were observed.

Conclusion: At night, the response was slower and required a higher dose because more severe airway obstruction was present on awakening. These results suggest that studies establishing the clinical dose of a β_2 -agonist or assessing the

equivalence of different formulations should be conducted in subjects with more severe reversible airway obstruction than is present during conventional daytime studies. (J Allergy Clin Immunol 2004;113:1058–62.)

Key words: Bronchodilator agents, adrenergic β -agonists, dose-response, bronchospasm

Conventional bronchodilator studies are typically conducted in subjects with mild to moderate airway obstruction during the daytime, when lung function is stable and reversible. Under these circumstances, even the lowest clinically used doses of short-acting β_2 -selective agonists produce a near maximum bronchodilator response.^{1–3} In other words, low doses achieve responses that are on the flat upper portion of the dose-response curve. As a consequence, it is often not possible to identify differences in response to different doses of the same formulation^{1–3} (eg, 1 vs 2 puffs delivered by metered dose inhaler [MDI]) or to different formulations that may deliver different quantities of drug to the airways^{4,5} (eg, MDI vs dry powder inhaler).

However, there may be some situations in which the dose delivered does make a difference. For example, whereas the US Food and Drug Administration–approved dose for albuterol MDI is 1 to 2 puffs every 4 to 6 hours,⁶ to obtain relief during an asthma attack, many patients appear to require >2 puffs⁷ and/or more frequent dosing.⁸ This suggests that a dose of 1 or 2 puffs no longer lies at the top of the dose-response curve and that a higher dose is needed when more intense airway obstruction is present.

Evidence from β -agonist dose-response studies using bronchoprovocation with histamine or methacholine,^{2,3,9,10} *in vitro* studies of isolated tracheal smooth muscle,¹¹ and *in vivo* studies in animals¹² support this concept. They indicate that the concentration of β -agonist required to overcome airway smooth muscle contraction is directly related to the intensity of the stimulus producing the contraction.

Nocturnal asthma is a potentially valuable, real-life model for studying responsiveness of intense airway obstruction to β -adrenergic drugs. It was our hypothesis that higher doses would be required to normalize lung function during nocturnal asthma than during conventional daytime studies, when less intense airway obstruction is present. We tested this hypothesis by comparing albuterol dose-response relationships when subjects awoke from asthma and when the same subjects were asymptomatic during the day.

From ^athe College of Pharmacy and Pulmonology Division, Department of Pediatrics; ^bthe Department of Statistics and ^cthe Division of Pulmonary and Critical Care Medicine, Department of Medicine, University of Florida; ^dPulmonary Associates of the Southeast, PC, Birmingham; and ^ethe Department of Pediatrics, Pediatric Allergy and Pulmonary Division, University of Iowa.

Supported in part by an investigator-initiated grant from the GlaxoSmithKline Respiratory Research Institute, Research Triangle Park, NC, by a pulmonary fellowship training grant from the American Lung Association, and by the National Institutes of Health General Clinical Research Center Grant #MO1 RR00082.

Disclosure of potential conflict of interest: L. Hendeles has a consultant arrangement with Kos Pharmaceuticals and Medicis Pharmaceuticals, has a stock or other equity ownership in Medicis Pharmaceuticals, receives grants or research support from GlaxoSmithKline, Merck, and Novartis, and is on the speaker's bureau for AstraZeneca, GlaxoSmithKline, Merck, and Novartis. R. Ahrens has consultant arrangements with AstraZeneca, Forest Laboratories, GlaxoSmithKline, IVAX, and Skye Pharma, and receives grants or research support from AstraZeneca and GlaxoSmithKline.

Received for publication January 13, 2004; revised March 25, 2004; accepted for publication March 26, 2004.

Reprint requests: Leslie Hendeles, PharmD, University of Florida, Health Sciences Center, PO Box 100486, 1600 SW Archer Road, Room MG-57, Gainesville, FL 32610-0486. E-mail: hendeles@cop.ufl.edu.

0091-6749/\$30.00

© 2004 American Academy of Allergy, Asthma and Immunology
doi:10.1016/j.jaci.2004.03.046

Abbreviations used

CRC: Clinical Research Center
MDI: Metered-dose inhaler

METHODS

Subject selection

Twenty-two subjects (10 females) age 18 to 44 years with a history of nocturnal asthma were selected. During the screening visit, their FEV₁ had to meet American Thoracic Society criteria for acceptability and reproducibility¹³ and had to be $\geq 60\%$ of predicted.¹⁴ To confirm bronchodilator responsiveness, nebulized albuterol (2.5 mg) was administered at 30-minute intervals until FEV₁ improved $\geq 15\%$ and to $\geq 80\%$ of predicted, or 3 doses had been inhaled. Subjects were excluded if they had any cardiac disease, recent tobacco use, or an upper respiratory tract infection. They used short-acting β_2 -agonists on an as-needed basis, and none were taking controller medications. The study was approved by the Institutional Review Board, and written informed consent was obtained.

Study protocol

Subjects were treated at the onset of nocturnal symptoms while sleeping in the Clinical Research Center (CRC) and during the day when they were asymptomatic in a randomized, unblind, crossover manner with at least a 48-hour interval between treatment periods. Before the screening visit and each treatment period, β_2 -agonists were withheld for 8 hours.

For the nocturnal treatment period, subjects were admitted to the CRC by 10:00 PM. If they did not wake from nocturnal asthma after sleeping there for 4 consecutive nights, they were withdrawn from the study. On admission, vital signs, oxyhemoglobin saturation, spirometry, and a 12-lead electrocardiogram were measured. An intravenous catheter was inserted for blood sampling. Before the first dose and 30 minutes after the last dose, oxyhemoglobin saturation, electrocardiogram, and blood samples for measurement of sodium, potassium, bicarbonate, lactic acid, and glucose plasma concentrations were obtained. Spirometry, blood pressure, and heart rate were measured before each dose and 30 minutes after the last dose.

Treatment with albuterol and data collection procedures were repeated in an identical manner during the randomized daytime period.

Medication administration

Albuterol was administered by Ventolin chlorofluorocarbon-MDI, 90 $\mu\text{g}/\text{puff}$ (GlaxoSmithKline, Research Triangle Park, NC), at 15-minute intervals through an InspirEase (Key Pharmaceuticals, Kenilworth, NJ) as follows: 1, 1, 2, 4, and 8 puffs, corresponding to 1, 2, 4, 8, and 16 cumulative puffs. Subjects were trained to inhale slowly, followed by a 10-second breath hold after each puff.

Statistical methods

Sample size and randomization. We estimated that 20 subjects would be required to detect a 1 puff difference between day and night treatments with a crossover design when $\alpha = 0.05$ and $\beta = 0.14$. Subjects were randomized in blocks of 4, to 1 of 2 sequences (day-night and night-day).

Data analysis. Each subject's personal best FEV₁ was identified as the highest value ever recorded after albuterol nebulizations on the screening visit or after randomized treatments. Then, each postdose FEV₁ was converted to a percent of personal best and modeled as a function of $\log_2$ dose (puffs) by using a random coefficients linear

TABLE I. Prestudy FEV₁ during the screening visit and capacity to reverse after bronchodilator among subjects who completed the study

Subject	Age (y)/sex	Screening day FEV ₁ , L (% predicted)	Personal best FEV ₁ , L (% predicted)*
1	19/M	3.43 (72)	4.19 (88)
2	32/F	1.59 (63)	2.31 (92)
3	34/F	1.52 (62)	2.71 (110)
4	24/F	2.53 (80)	3.04 (96)
5	28/F	2.36 (79)	3.29 (110)
6	32/F	1.73 (60)	3.07 (107)
9	35/M	2.99 (75)	3.92 (99)
10	22/F	2.15 (63)	3.29 (97)
11	21/F	2.49 (69)	3.07 (85)
12	37/M	2.41 (65)	3.27 (88)
15	18/F	2.37 (73)	3.05 (97)
16	28/M	3.20 (72)	3.93 (88)
17	19/M	2.82 (62)	3.55 (78)
21	22/M	2.92 (63)	4.34 (93)
22	23/M	2.81 (60)	4.28 (91)
Mean	26.3	2.50 (68)	3.40 (95)
SD	6.4	0.57 (6.8)	0.60 (9.2)

*The highest FEV₁ ever recorded for a subject after 1 to 3 albuterol nebulizations during the screening visit or during day or night treatments.

regression analysis.¹⁵ This method indicates whether there is a significant difference in slopes and intercepts between day and night treatments. The slope and intercept for each subject was used to interpolate the number of puffs required to achieve 80% of the subject's personal best FEV₁ during each treatment period (primary outcome). This endpoint represents a good response to β -agonists in the algorithm for the management of an acute exacerbation at home in the National Institutes of Health Guidelines.¹⁶ The difference in this endpoint between treatments was analyzed with the Mann-Whitney Test. A paired *t* test was used to compare the maximum improvement in FEV₁ and maximum FEV₁ achieved during each treatment period.

Within-treatment changes from the predose value for systemic effects were compared by an ANOVA for repeated measures. Results were expressed as means $\pm$ SDs, means (95% CIs), or median (25th, 75th percentiles), as appropriate. Statistical significance was accepted at $P < .025$ when the *t* test was used twice and $P < .05$ for all other comparisons.

RESULTS

Of the 22 subjects randomized to treatment, 15 (8 females) age 18 to 37 years completed the study. Five subjects were excluded because they did not wake from asthma symptoms in the CRC. A sixth subject was excluded because of an asthma exacerbation, and a seventh subject was excluded because of the presence of barbiturates in his urine. At the prestudy screening visit, all of the subjects who completed the study had moderate airway obstruction with the capacity to achieve normal or near normal lung function after albuterol (Table I).

On admission to the CRC, all subjects were asymptomatic. The mean $\pm$ SD FEV₁ was 64% $\pm$ 11% predicted. On awakening from asthma symptoms, severe airway

TABLE II. Response to 16 cumulative puffs of albuterol for each subject completing both day and night treatments

Subject	Predose FEV ₁ , L (% predicted)*		Maximum improvement in FEV ₁ , L		Maximum postdose FEV ₁ , L (% predicted)*	
	Day	Night	Day	Night	Day	Night
1	2.99 (62)	1.58 (33)	1.05	1.76	4.00 (85)	3.34 (70)
2	1.52 (60)	1.37 (54)	0.75	0.94	2.23 (90)	2.31 (92)
3	1.69 (68)	1.28 (52)	1.02	1.33	2.71 (110)	2.61 (105)
4	2.52 (79)	1.36 (43)	0.48	1.68	2.95 (95)	3.00 (96)
5	2.48 (83)	2.15 (72)	0.77	1.14	3.25 (109)	3.29 (110)
6	2.19 (76)	1.03 (36)	0.88	1.75	3.07 (107)	2.78 (97)
9	3.05 (77)	1.97 (50)	0.76	0.64	3.81 (96)	2.61 (66)
10	1.90 (56)	1.41 (42)	1.22	1.88	3.12 (92)	3.29 (97)
11	2.61 (73)	1.31 (36)	0.38	1.21	2.99 (83)	2.52 (70)
12	2.23 (59)	1.33 (36)	0.55	0.72	2.78 (75)	2.05 (55)
15	2.24 (69)	0.85 (26)	0.77	2.06	3.01 (93)	2.91 (90)
16	2.98 (66)	2.11 (47)	0.65	1.06	3.63 (81)	3.17 (71)
17	3.22 (70)	2.82 (62)	0.30	0.73	3.52 (77)	3.55 (78)
21	2.60 (56)	2.18 (47)	0.96	1.63	3.47 (74)	3.81 (82)
22	2.80 (59)	1.37 (29)	1.14	2.10	3.94 (83)	3.47 (74)
Mean	2.47 (68)	1.61 (44)	0.78	1.38	3.23 (90)	2.98 (84)
SD	0.51 (8.7)	0.53 (12)	0.27	0.50	0.50 (12)	0.50 (15)
P value	.0001		.0001		.02	

*Percent predicted based on the equations of Morris et al.¹⁴

obstruction was present; FEV₁ decreased to a mean of 44% $\pm$ 12% predicted (Table II). Oxyhemoglobin saturation was normal on awakening, 97 $\pm$ 1%, and not significantly different from the values measured at other time points.

As a result of the lower predose value at the onset of nocturnal symptoms, the maximum improvement in FEV₁ was significantly larger at night (1.38 $\pm$ 0.50 L) than during the day (0.78 $\pm$ 0.27 L; Table II) and correlated inversely with the predose FEV₁ ($r = 0.83$). In contrast, the mean maximum postdose FEV₁ achieved after albuterol was significantly lower at night (84% $\pm$ 15% predicted) than at the end of the daytime treatment period (90% $\pm$ 12% predicted; $P = .02$; Table II).

Also, because of the lower FEV₁ on awakening from asthma, the mean (95% CI) slope of the log₂ dose-response curve was steeper during the nocturnal episode (7.4 [5.8, 9.0] % personal best/doubling cumulative puffs) than during the day (4.1 [3.3, 4.9]/doubling cumulative puffs; $P = .002$) and was shifted to the right (Fig 1). As a consequence, the median (25th, 75th percentiles) dose estimated to achieve 80% of the subject's personal best FEV₁ was 5 (1, 19) puffs at night, but only 0.4 (<0.25, 2) puffs during the day ($P = .02$; Fig 2). The response to albuterol was slower during the night; the median time (25th, 75th percentiles) required to achieve this endpoint was 47 (21, 90) minutes at night but only 10 (0.2, 42) minutes during the day ($P = .002$).

The predose mean serum potassium concentration was significantly lower at night than the day, but treatment with albuterol did not affect this or any other metabolic or cardiovascular endpoint (Table III).

DISCUSSION

The results of this study indicate that during nocturnal asthma, response to albuterol was slower, was less

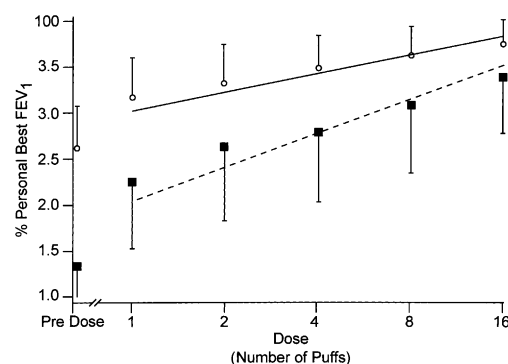


FIG 1. The mean $\pm$ SD FEV₁ (expressed as a percentage of personal best) measured 15 minutes after each log₂ cumulative dose during nocturnal asthma (■) and during the day (○) in the 15 subjects who completed both treatments. The predose values are indicated on the y-axis as a reference and are not part of the dose-response curves.

complete, and required a larger number of puffs to normalize lung function than during the day when subjects had asymptomatic airway obstruction. These differences were most likely a result of the severe airway obstruction on awakening from nocturnal asthma.

Our results are supported by a previous study of epinephrine in 5 subjects with nocturnal asthma. Barnes et al¹⁷ found a steeper dose-response curve to increasing cumulative doses of intravenous epinephrine at 4 AM than at 4 PM. Also, during the next 24 hours, without a washout period, they found a greater increase in peak expiratory flow after a single dose of 2 puffs of epinephrine MDI (560 μ g) at 4 AM than at other times.

The characteristics of response to albuterol seen in this study are compatible with concepts of functional antagonism. *In vitro* studies of tracheal or bronchial strips¹¹ and *in vivo* studies in animals¹² indicate that the dose-response

TABLE III. Systemic effects during day and night treatment periods

	Mean (SD)			
	Day		Night	
	Predose	Postdose*	Predose	Postdose*
Cardiovascular effects				
Heart rate (bpm)	84 (14)	82 (10)	87 (15)	83 (11)
Mean arterial blood pressure (mm Hg)	84 (7)	87 (8)	92 (13)	96 (12)
Diastolic blood pressure (mm Hg)	69 (8)	68 (7)	76 (13)	73 (7)
Metabolic effects (normal range)				
K ⁺ (3.2-5.5 mEq/L)	4.4 (0.5)	4.2 (0.6)	3.8 (0.3)†	3.6 (0.3)
Na ⁺ (135-149 mEq/L)	139 (2.6)	139 (2.0)	141 (2.6)	141 (2.6)
HCO ₃ ⁻ (24-32 mEq/L)	24 (2.2)	23 (2.8)	24 (2.1)	24 (2.7)
Lactic acid (0.67-2.47 mEq/L)	1.2 (0.6)	1.4 (0.4)	1.0 (0.3)	1.1 (0.5)
Glucose (65-110 mg/dL)	92 (15)	97 (14)	94 (13)	99 (10)

*Measured 30 minutes after a cumulative dose of 16 puffs.

†P = .01 for comparison of day and night predose means.

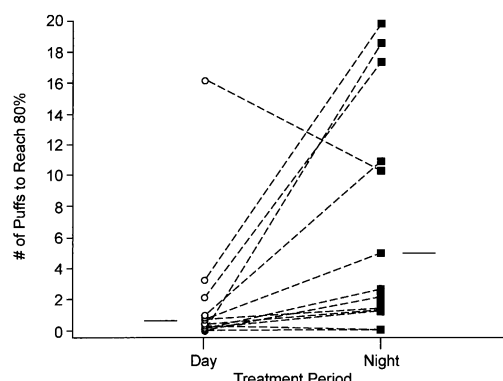


FIG 2. The estimated dose of albuterol (number of puffs) required to achieve 80% of the subject's personal best FEV₁ during nocturnal asthma (■) and during the day (○). Each data point was estimated from the subject's log₂ dose-response curve. The medians (—) were 5 puffs at night and 0.4 puffs during the day (P = .01).

curve is shifted to the right when higher concentrations of airway smooth muscle constrictants are used, and that the maximum effect of the β -agonist is significantly lower. Also, it is possible that changes at the level of the β_2 -adrenergic receptor,¹⁸ inflammation,¹⁹ and decreased cortisol production²⁰ could have contributed to the altered response at night.

The results of this study have an important implication. Conventional daytime bronchodilator studies can be misleading when establishing the therapeutic dose of a new β_2 -agonist or therapeutic equivalence of different methods of delivering the same drug. When response is on the flat upper portion of the β -agonist dose-response curve, different doses of the same formulation or formulations that deliver different amounts of drug to the airway will produce equivalent, near maximal bronchodilator responses. However, when functional antagonism of β -agonist effect is greater, either during more severe airway obstruction as in the current study or when bronchospasm is induced by a constrictant such as

methacholine or histamine, the same doses will then be on the rising part of the dose-response curve. Under these circumstances, differences in the dose administered or in the amount of drug delivered to the airways by different formulations²¹⁻²³ are more likely to yield differences in clinical response.

When a cumulative dosing scheme is used, as in the current study, FEV₁ response is a function of both the dose administered and the length of time since the subject awoke. As a result, it is not possible to determine pharmacodynamic parameters such as the dose required to produce 50% of the maximum response or dose-potency ratios accurately. These estimates assume that response is exclusively a function of dose.²⁴ It is for this reason that we reported the slopes of the dose-response curves without attempting to calculate the dose required to produce 50% of the maximum response.

In conclusion, during nocturnal asthma, the response to inhaled albuterol is slower, is less complete, and requires a higher dose to normalize lung function than during the day because of more severe airway obstruction on awakening. These results are compatible with the concept of functional antagonism. They indicate that conventional daytime bronchodilator studies can fail to detect important differences in efficacy of inhaled β -agonists or formulations because the dose-response curve is relatively flat. Thus, the nocturnal study design offers a more clinically relevant alternative to conventional daytime bronchodilator studies or those using bronchoprotection.

We thank Leslie Molino, RN, who recruited the subjects and performed the screening visits, Charles Church, RN, who skillfully treated the subjects and collected the data at night in the CRC, Kathy Rice for preparing the tables and word processing the many drafts, and John Jenne, MD, for his helpful comments.

REFERENCES

1. Fishwick D, Bradshaw L, Macdonald C, Beasley R, Gash D, Bengtsson T, et al. Cumulative and single-dose design to assess the bronchodilator effects of β_2 -agonists in individuals with asthma. *Am J Respir Crit Care Med* 2001;163:474-7.

2. Blake KV, Hoppe M, Harman E, Hendeles L. Relative amount of albuterol delivered to lung receptors from a metered-dose inhaler and nebulizer solution: bioassay by histamine bronchoprovocation. *Chest* 1992;101:309-15.
3. Ahrens RC, Harris JB, Milavetz G, Annis L, Ries R. Use of bronchial provocation with histamine to compare the pharmacodynamics of inhaled albuterol and metaproterenol in patients with asthma. *J Allergy Clin Immunol* 1987;79:876-82.
4. Mellén A, Arvidsson P, Palmqvist M, Lötvald J. Equivalent bronchodilation with salbutamol given via pMDI or Turbuhaler. *Am J Respir Crit Care Med* 1999;159:1663-5.
5. Hultquist C, Ahlström H, Kjellman NI, Malmqvist LÅ, Svenonius E, Melin S. A double blind comparison between a new multidose powder inhaler (Turbuhaler) and metered dose inhaler in children with asthma. *Allergy* 1989;44:467-70.
6. Ventolin product information. In: Physicians' desk reference. Montvale (NJ): Medical Economics Co; 2001. p. 1680.
7. Idris AH, McDermott MF, Raucci JC, Morrabel A, McGorray S, Hendeles L. Emergency department treatment of severe asthma: metered-dose inhaler plus holding chamber is equivalent in effectiveness to nebulizer. *Chest* 1993;103:665-72.
8. Windom HH, Burgess CD, Crane J, Pearce N, Kwong T, Beasley R. The self-administration of inhaled beta agonist drugs during severe asthma. *N Z Med J* 1990;103:205-7.
9. Parameswaran KN, Inman MD, Ekholm BP, Morris MM, Summers E, O'Byrne PM, et al. Protection against methacholine bronchoconstriction to assess relative potency of inhaled β_2 -agonist. *Am J Respir Crit Care Med* 1999;160:354-7.
10. Creticos PS, Adams WP, Petty BG, Lewis LD, Singh GJP, Khattignavong AP, et al. A methacholine challenge dose-response study for development of a pharmacodynamic bioequivalence methodology for albuterol metered-dose inhalers. *J Allergy Clin Immunol* 2002;110:713-20.
11. Roux FJ, Grandordy B, Douglas JS. Functional and binding characteristics of long-acting β_2 -agonists in lung and heart. *Am J Respir Crit Care Med* 1996;153:1489-95.
12. Jenne JW, Shaughnessy TK, Druz WS, Manfredi CJ, Vestal RE. In vivo functional antagonism between isoproterenol and bronchoconstrictants in the dog. *J Appl Physiol* 1987;63:812-9.
13. American Thoracic Society. Standardization of spirometry, 1994 update. *Am J Respir Crit Care Med* 1995;152:1107-36.
14. Morris JF, Koski A, Johnson LC. Spirometric standards for healthy nonsmoking adults. *Am Rev Respir Dis* 1971;103:57-67.
15. Statistical analysis system. Version 8. Cary (NC): SAS Institute; 2000.
16. National Asthma Education and Prevention Program expert panel report 2: guidelines for the diagnosis and management of asthma: clinical practice guidelines. Bethesda (MD): National Institutes of Health; 1997p. 60. Publication 97-4051.
17. Barnes PJ, Fitzgerald GA, Dollery CT. Circadian variation in adrenergic responses in asthmatic subjects. *Clin Sci* 1982;62:349-54.
18. Szefer SJ, Ando R, Cicutto LC, Surs W, Hill MR, Martin RJ. Plasma histamine, epinephrine, cortisol, and leukocyte β -adrenergic receptors in nocturnal asthma. *Clin Pharmacol Ther* 1991;49:59-68.
19. Martin RJ, Cicutto LC, Smith HR, Ballard RD, Szefer SJ. Airways inflammation in nocturnal asthma. *Am Rev Respir Dis* 1991;143:351-7.
20. Barnes P, FitzGerald G, Brown M, Dollery C. Nocturnal asthma and changes in circulating epinephrine, histamine, and cortisol. *N Engl J Med* 1980;303:263-7.
21. Bondesson E, Friberg K, Soliman S, Löfdahl CG. Safety and efficacy of a high cumulative dose of salbutamol inhaled via Turbuhaler or via a pressurized metered-dose inhaler in patients with asthma. *Respir Med* 1998;92:325-30.
22. Ekström T, Andersson AC, Skedinger M, Lindblad C, Ståhl E. Dose potency relationship of terbutaline inhaled via Turbuhaler or via a pressurized metered dose inhaler. *Ann Allergy Asthma Immunol* 1995;74:328-32.
23. Carlsson LG, Arweström E, Frierg K, Källén A, Lunde H, Löfdahl CG. Efficacy of cumulative doses of salbutamol administered via Turbuhaler or Diskhaler in patients with reversible airway obstruction. *Allergy* 1998; 53:712-5.
24. Finney DJ. Statistical methods in biological assay. 3rd ed. London: Charles Griffin & Co; 1978. p. 211-2.