

Pulmonary function response to EDTA, an additive in nebulized bronchodilators

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Background: Some nebulized bronchodilator solutions contain additives, such as EDTA, benzalkonium chloride (BAC), or both.

Objective: Although BAC-induced bronchoconstriction has been well documented in patients with asthma, there is no information on the effects of EDTA on FEV₁ when inhaled in the amounts that would be administered during emergency department treatment of asthma.

Methods: Eighteen subjects with stable asthma and airway responsiveness to methacholine were randomly assigned to inhale up to four 600- μ g nebulized doses of EDTA, BAC (positive control), and normal saline (placebo) in a double-blind crossover manner on separate days. FEV₁ was measured 15 minutes after each dose. Treatments were repeated every 20 minutes until FEV₁ decreased by 20% or greater or a maximum of 4 doses were administered.

Results: Mean $\pm$ SD maximum percent decrease in FEV₁ was 1.8% $\pm$ 5.8% after EDTA, 16.6% $\pm$ 13.9% after BAC, and 3.6% $\pm$ 8.2% after placebo ($P < .001$); there was no significant difference between EDTA and placebo.

Conclusion: The amount of EDTA contained in maximum recommended doses of nebulized bronchodilators does not induce bronchospasm. In contrast, BAC induces clinically important bronchospasm, which could decrease the efficacy of a bronchodilator during an emergency. (*J Allergy Clin Immunol* 2001;107:68-72.)

Key words: EDTA, benzalkonium compounds, albuterol

Nebulizer solutions available throughout the world to treat asthma and chronic obstructive pulmonary disease often contain additives, such as EDTA, benzalkonium chloride (BAC), or a combination of the two.^{1,2} BAC, the most common additive in the United States, is the antibacterial preservative in nonsterile multidose 0.5% albuterol inhalant solutions (all US manufacturers), nonsterile screwcap unit-dose 0.083% albuterol nebulizer solutions (Schering-Plough, Madison, NJ and Warrick, Union, NJ), and metaproterenol nonsterile multidose

Abbreviations used

BAC: Benzalkonium chloride

ED: Emergency department

inhalant solutions (all US manufacturers).³ EDTA, on the other hand, does not inhibit microbial growth but is added to metaproterenol (all US manufacturers) and some albuterol sterile-filled unit-dose inhalant solutions (Dey LP, Napa, Calif) to chelate metal contaminants and thus prevent solution discoloration.³ Ipratropium nebulizer solution (Boehringer Ingelheim) containing both BAC and EDTA is available in more than 50 countries, whereas the formulation available in the United States and the United Kingdom does not contain either additive.²

BAC-induced bronchospasm has been well documented in patients with asthma with an FEV₁ of greater than 60%.⁴⁻⁹ It occurs within 5 minutes after inhalation, acts cumulatively on repeated dosing, and persists for over an hour.⁸ These pharmacodynamic properties are likely to be important during repeated dosing of bronchodilators containing BAC, as occurs in the emergency department (ED) or hospital treatment of acute asthma. In a bronchoprovocation study the threshold dose of BAC required to decrease FEV₁ by 20% or greater was about 300 μ g.⁸

EDTA-induced airway obstruction has been well documented in animals but not in human subjects. In canines EDTA aerosol challenge decreased lung function in 5 minutes,^{10,11} was sustained for at least 25 minutes, and rapidly reversed after albuterol.¹² The only reported study in human subjects was a brief report in which 6 subjects who experienced paradoxical bronchospasm to ipratropium containing 0.25 mg/mL BAC and 0.5 mg/mL EDTA subsequently underwent bronchoprovocation with both ingredients on separate days.¹³ Airway obstruction appeared within minutes after inhaled EDTA challenge and persisted for at least an hour. The mean EDTA concentration required for a decrease in FEV₁ of 20% or greater in these 6 subjects, 2.4 mg/mL, was 2- to 4-fold the 0.1 to 0.5 mg/mL EDTA concentrations available in bronchodilators marketed in the United States. However, it is unclear how much EDTA was delivered to these subjects because characteristics of the delivery method used in the study were not described. It is possible that these investigators delivered a much larger amount of EDTA

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Supported in part by a grant from Dey LP, Napa, California.

Conflict of interest: Dr Hendeles serves on a National Pharmacy Advisory Board to Dey LP, Napa, California, the sponsor of this study.

Received for publication July 3, 2000; revised August 8, 2000; accepted for publication September 14, 2000.

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0091-6749/2001 \$35.00 + 0 1/81/111591

doi:10.1067/mai.2001.111591

than would be inadvertently delivered by disposable nebulizer during ED treatment with an albuterol product containing this ingredient.

The National Asthma Education and Prevention Program recommends 2.5 to 5 mg of nebulized albuterol administered every 20 minutes for the first hour during treatment of acute asthma.¹⁴ Thus if the 0.083% screw-cap albuterol product is used, a patient would inhale a solution containing up to 1800 µg of BAC in the first hour of ED treatment. It is currently unknown whether inhaling a solution containing the same amount of EDTA is enough to cause airway obstruction. Because a previous study indicated a positive relationship between airway responsiveness to inhaled histamine and the occurrence of BAC-induced bronchospasm,⁸ we only selected subjects who were very responsive to inhaled methacholine. The treatments were administered in the same manner that an albuterol nebulizer solution would be given during ED treatment of acute asthma. The hypothesis of this study was that EDTA would induce bronchospasm in a dose-dependent manner and that the response would be greatest in subjects with greater airway responsiveness to methacholine.

METHODS

Subjects

Nonsmoking adult subjects with a history of stable asthma and a baseline FEV₁ of 65% or greater of predicted value for height, age, sex, and race¹⁵ were recruited for this study. At the initial screening visit, all subjects had to demonstrate airway responsiveness to methacholine with a PC₂₀ of 2 mg/mL or less. Subjects with a history of life-threatening asthma or anaphylaxis were excluded from the study. Other reasons for exclusion were an ED visit or hospitalization for asthma within 3 months, the reported use of oral corticosteroids within 3 months, or a respiratory tract infection during or within 6 weeks before the study. Women could neither be pregnant nor lactating and had to be using an adequate method of contraception. Before each study visit, subjects withheld short-acting β-adrenergic bronchodilators for at least 6 hours, long-acting β-adrenergic bronchodilators for a minimum of 48 hours, slow-release theophylline for 48 hours, short-acting antihistamines for 4 days, leukotriene modifiers for 48 hours, and caffeine for 8 hours. The study was approved by the University of Florida Health Science Center Institutional Review Board, and witnessed written informed consent was obtained from each subject.

Study design

Subjects were randomly assigned to inhale 3 mL of 0.9% sodium chloride injection containing 600 µg of BAC (benzalkonium chloride 50% solution, NF; Spectrum, Gardena, Calif), 3 mL of 0.9% sodium chloride injection containing 600 µg of EDTA (EDTA powder, USP; Spectrum), and 3 mL of placebo solution (3 mL of 0.9% sodium chloride injection, USP; Baxter Healthcare Corp, Round Lake, Ill) every 20 minutes in a double-blind crossover manner on separate days. Treatment solutions were prepared in advance by the Investigational Drug Service at Shands Hospital (Gainesville, Fla) by using aseptic technique and stored at 2°C to 8°C in polypropylene unit-dose syringes. Solutions were allowed to warm to room temperature immediately before use and were discarded if unused. Each 3-mL dose was inhaled by using normal tidal breathing through the Pari LC Star jet nebulizer (Pari Respiratory Equipment, Inc, Midlothian, Va) powered by a Proneb-Turbo compressor (Pari Respiratory Equip-

TABLE I. Pulmonary function and airway responsiveness to methacholine measured during the screening visit for the 18 subjects who completed the study

Sex	Age (y)	FEV ₁ , L (% predicted)	PC ₂₀ (mg/mL)
M	23	3.12 (71)	0.3
F	47	2.70 (89)	1.0
M	26	4.17 (86)	0.7
F	41	2.76 (76)	0.6
F	33	3.15 (103)	2.2
F	40	2.64 (87)	0.2
M	25	3.49 (77)	0.3
F	29	2.65 (96)	0.2
F	23	3.12 (94)	1.3
F	29	2.76 (73)	0.2
F	18	3.72 (105)	0.4
F	43	2.16 (85)	0.6
M	25	2.98 (69)	1.4
F	20	2.73 (81)	0.6
F	20	3.27 (94)	0.7
F	25	2.77 (79)	0.1
M	19	5.09 (95)	0.6
F	19	3.14 (88)	0.4
Mean	28	3.13 (86)	0.5*
SD	9	0.67 (11)	0.4†

*Geometric mean.

†Geometric SD.

ment, Inc) until nebulization ceased (8-10 minutes). To reduce potential variability associated with using multiple nebulizers, the nebulizer output of each unit was confirmed as 0.24 to 0.25 mL/min by using gravimetric analysis. Spirometry was performed at the beginning of the study day and 15 minutes after inhalation of each dose began. Treatments were repeated every 20 minutes until FEV₁ decreased by at least 20% or a maximum of four 3-mL doses were administered (ie, a cumulative volume of solution containing 2400 µg of additive). Subjects were given 180 µg of albuterol from a metered-dosed inhaler to reverse bronchoconstriction if it occurred or on request. The albuterol dose was repeated, if necessary, 20 minutes later. Subjects were discharged from the Asthma Research Laboratory when FEV₁ returned to within 10% of that day's baseline.

Spirometry

FEV₁ was measured with a KoKo Pneumotach Spirometer (Pulmonary Data Service, Louisville, Colo) that was calibrated daily. Spirometry was performed at the beginning of each visit to ensure that FEV₁ was 65% or greater of predicted value and within ±10% of the value measured at the screening visit. The visit was rescheduled if one of these criteria was not met. All spirometric maneuvers were performed by using a modification of the American Thoracic Society acceptability and reproducibility criteria.¹⁶ To minimize within-subject spirometry variability, our goal was to have the 2 highest FEV₁ values (from 3 acceptable efforts) within 5% of each other with no more than a 100-mL difference. This goal was achieved during 175 (91%) of 192 sets of spirometry.

Methacholine challenge

Airway responsiveness was determined at the screening visit by using the Rosenthal method.¹⁷ Briefly, methacholine was inhaled through the KoKo DigiDoser Dosimeter (Pulmonary Data Service) by taking 5 full inspirations from functional residual capacity to total lung capacity. Each subject received 2-fold increasing concentrations of methacholine (0.312, 0.62, 1.25, and 2.5 mg/mL) until the FEV₁ decreased by at least 20% from baseline or 2.5 mg/mL had been admin-

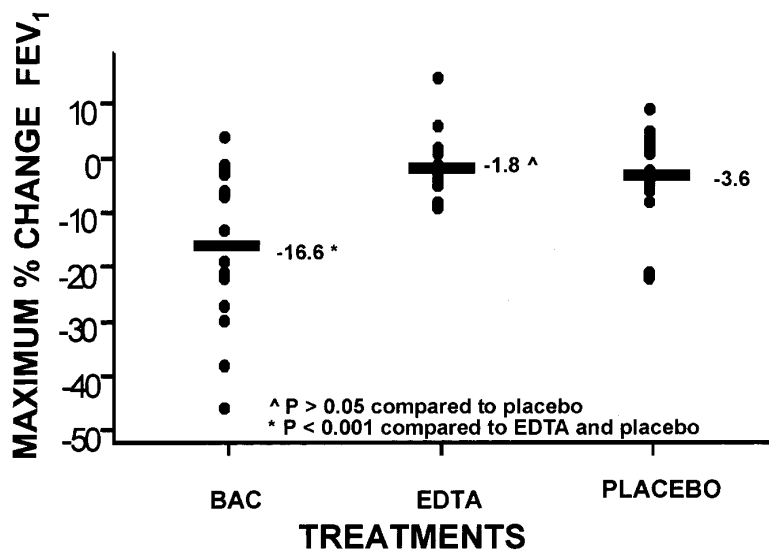


FIG 1. Maximum percent change in FEV₁ after inhaling one to four 600- μ g doses of BAC, EDTA, or matched placebo in each of the 18 subjects.

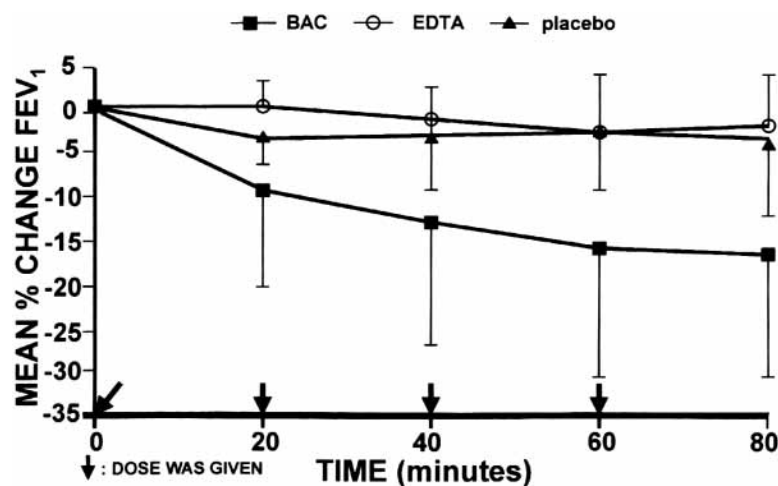


FIG 2. Time course of mean change in FEV₁ in 18 subjects that inhaled one to four 600- μ g doses of BAC, EDTA, or matched placebo.

istered. After the methacholine challenge, albuterol from a metered-dose inhaler was given to reverse bronchoconstriction until the subject's FEV₁ returned to at least 90% of prechallenge baseline values. All subjects had to have a methacholine PC₂₀ of 2 mg/mL or less. Methacholine (Provocholine; InSource, Inc, Bland, Va) solutions were prepared by the Investigational Drug Service at Shands Hospital by using aseptic technique. Solutions were stored frozen (-20°C to -7°C) in polypropylene unit-dose syringes and allowed to warm to room temperature immediately before use. Unused solutions were discarded.

Data analysis

The primary outcome measure, the maximum percent change in FEV₁ from predrug baseline FEV₁, was evaluated by using repeated-measures ANOVA with a Bonferroni multiple comparison test.

Least-squares regression analysis was used to investigate the relationship between the maximum percent drop in FEV₁ and the predictor variable, log methacholine PC₂₀. Also, a nonparametric distribution analysis with Kaplan-Meier estimates was performed to describe the probability of a significant change in FEV₁ (ie, $\geq 20\%$ drop) occurring after inhaling 1, 2, 3, or 4 doses of study drug. All data analysis was performed by using Minitab release 12 statistical software (Minitab, Inc, State College, Pa).

RESULTS

Eighteen adult subjects with mild resting bronchospasm and marked airway responsiveness to methacholine completed all 3 treatment arms of the study

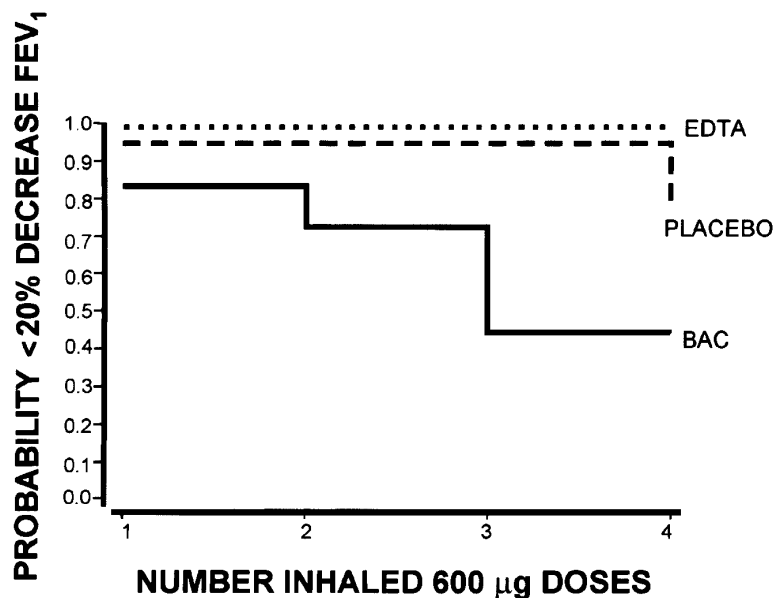


FIG 3. The probability of experiencing a less than 20% decrease in FEV₁ after each cumulative dose of BAC, edetate disodium (EDTA), or matched placebo.

(Table I). All subjects were using as-needed inhaled β_2 -sympathomimetics, 6 were taking inhaled corticosteroids, and 2 were taking a leukotriene modifier. Concurrent medications were withheld before all study visits, as outlined in the "Methods" section.

None of the subjects experienced a decrease in FEV₁ of greater than 10% after inhaling all four 600- μ g doses of EDTA (Fig 1). The time course of effect after EDTA was not different from placebo (Fig 2). The mean maximum decrease in FEV₁ was $1.8\% \pm 5.8\%$ after EDTA, $16.6\% \pm 13.9\%$ after BAC, and $3.6\% \pm 8.2\%$ after placebo ($P < .001$). Interestingly, one subject experienced a 23% drop in FEV₁ after inhaling a single 3-mL dose of placebo (normal saline), and a second subject experienced a 22% drop in FEV₁ after the fourth and final placebo dose (Fig 1). Normal saline control solutions were confirmed to be free of any BAC contaminant.¹⁸ In contrast, there was a decrease in FEV₁ after BAC that was dependent on dose, time, or an interaction of both (Fig 2). Three (17%) of 18 subjects experienced a drop of 20% or greater in FEV₁ after inhaling just one 600- μ g dose of BAC (Fig 1). After inhaling two 600- μ g BAC doses, 2 additional subjects had experienced unacceptable bronchoconstriction. One of these subjects had a 47% drop in FEV₁, whereas the other subject experienced a 14% drop and elected not to continue further dosing because of discomfort. Five additional subjects experienced a drop of 20% or greater in FEV₁ after inhaling a third 600- μ g dose of BAC. Cumulatively, 10 (55%) of 18 subjects had at least a 20% decrease in FEV₁ (Fig 3). Only 8 subjects had a less than 10% decrease after inhaling four 600- μ g doses of BAC. The probability of having a drop of 20% or greater in FEV₁ is significantly larger for BAC ($P < .0001$) compared with either EDTA or placebo (Fig 3).

There was no significant correlation between the maximum percent drop in FEV₁ caused by either EDTA ($r^2 = 0.02$) or BAC ($r^2 = 0.25$) and the log methacholine PC₂₀.

DISCUSSION

The results of this study indicate that the amount of EDTA contained in some 5-mg nebulized albuterol treatments (ie, 600 μ g) is not enough to induce clinically important bronchospasm, even after repeated dosing, as would occur in the ED treatment of asthma. Larger quantities of EDTA than we administered are unlikely to be administered. In contrast, BAC induced clinically important bronchospasm in 10 of 18 subjects. Because this was a cumulative dose design, it is not possible to determine whether the adverse effect is dependent on dose, time, or an interaction of both.

The results of this study for EDTA are different from those reported by Beasley et al.¹³ The most likely explanation is that they delivered a much larger amount. They used higher concentrations (2.5-10 mg/mL) of EDTA delivered by a different device (Inspiron Nebulizer).

Our study found no correlation between airway responsiveness to methacholine and bronchoconstrictive response to BAC. Previous investigations enrolled subjects with histamine PC₂₀ values of 0.11 to 7.8 mg/mL,⁸ whereas 2 mg/mL was the highest value in our subjects. It is possible that we missed a correlation because of the narrow range of PC₂₀ values in our subjects.

Although it was not the focus of the present study, our results confirm the observations of other investigators that BAC caused significant bronchoconstriction in asthmatic subjects when inhaled in doses included in some nebulized bronchodilators.⁸ Admittedly, evidence

demonstrating that the presence of BAC in a bronchodilator leads to paradoxical bronchoconstriction is circumstantial. However, the potential for reduced response to the bronchodilator does exist. It is illogical and potentially dangerous to use a nebulized bronchodilator containing a potent bronchoconstrictor like BAC in any clinical situation, especially when additive free bronchodilators are available.

In conclusion, the amount of EDTA contained in a 5-mg dose of albuterol is unlikely to induce bronchospasm, even after frequent doses at short intervals, as would occur during the ED treatment of acute asthma. In contrast, the same amount of BAC contained in the screwcap unit-dose albuterol product is sufficient to induce clinically important bronchospasm and may impair the therapeutic response during a medical emergency.

Nebulizers and compressors were provided by Pari Respiratory Equipment, Inc, Midlothian, Virginia. EDTA and BAC powder were provided by Dey LP, Napa, California. Rhonda Cooper-DeHoff, PharmD, Investigational Drug Service of Shands Hospital at the University of Florida prepared blinded sterile unit dose solutions. Fu-Bao Ji, Whei-Mei Wu, and Intira Coowanitwong provided technical support for sample identification.

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