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Inhibition of airway hyperreactivity by oxybutynin chloride in subjects with cervical spinal cord injury

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Objective: To further investigate mechanisms of airway hyperreactivity among subjects with chronic cervical spinal cord injury (SCI), we assessed airway responsiveness to aerosolized methacholine and histamine in subjects receiving chronic oxybutynin chloride therapy, and compared the findings with those not receiving the agent.
Methods: Twenty-five male subjects with cervical SCI participated in this study; 12 were maintained on oral oxybutynin chloride and 13 served as age-matched controls. Six of the 12 subjects receiving oxybutynin were challenged with aerosolized methacholine, and six with histamine; seven of the 13 control subjects were challenged with aerosolized methacholine and the remaining six with histamine.
Results: All 13 control subjects and all six oxybutynin/histamine subjects exhibited a significant bronchoconstrictor response (PC20 $<$ 8 mg/ml), whereas mean PC20 values for the oxybutynin/methacholine group were $\geqslant$25 mg/ml.
Conclusion: Our finding that the bronchoconstrictor effects of methacholine were blocked by oxybutynin chloride while those of histamine were not suggests that oxybutynin acts primarily through anticholinergic pathways rather than by causing generalized airway smooth muscle relaxation.

Keywords: ditropan; tetraplegia; histamine; methacholine; broncho-provocation

Introduction

Oxybutynin chloride (Ditropan) has been widely used therapeutically in humans to treat gastro-intestinal and urinary tract disorders characterized by smooth muscle hypermotility.[1,2] Pharmacological action, as assessed in animal studies involving the gastro-intestinal tract, uterus, seminal vesicle, bladder and detrusor, has been attributed to direct potent musculotropic antispasmodic action, probably reflecting a combination of local anesthetic properties and moderate anticholinergic and antihistamine activity.[1,3,4] In a study of pulmonary effects, pretreatment of conscious guinea pigs with subcutaneous oxybutynin blocked carbachol-induced, respiratory-associated generalized collapse, and pretreatment by intravenous administration in anesthetized animals inhibited methacholine-induced bronchoconstriction.[5] Whether oxybutynin causes relaxation of human airway smooth muscle or attenuates experimentally-induced bronchoconstriction has not been determined.

Previously we demonstrated that approximately 45% of subjects with chronic cervical spinal cord injury (SCI) experienced significant bronchodilatation following inhalation of either metaproterenol sulfate or ipratropium bromide.[6,7] Furthermore, approximately 80% of those subjects displayed airway hyperresponsiveness following inhalation of methacholine, histamine and/or distilled water.[8–10] Airway hyperresponsiveness induced by methacholine, but not that induced by histamine, was blocked in subjects chronically maintained on baclofen, a GABA-B agonist with central nervous system inhibitory neurotransmitter and anticholinergic activity.[8,9] The objective of this study was to further elucidate mechanisms of airway hyperreactivity in subjects with cervical SCI, and to determine whether oxybutynin affects airway smooth muscle function in humans. We therefore assessed airway hyperresponsiveness to methacholine and histamine in subjects receiving chronic oxybutynin therapy, and compared the findings with those from subjects with cervical SCI not undergoing such treatment.

Subjects and methods

The study group consisted of 25 males with traumatic chronic cervical SCI: 12 who were chronically

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maintained on oral oxybutynin chloride (>1 year) and 13 age-matched subjects who were not receiving this agent. Six of the 12 subjects receiving oxybutynin were challenged with aerosolized methacholine, and six with histamine; seven of the 13 control subjects were challenged with aerosolized methacholine, and the remaining six with histamine. Oxybutynin chloride is commonly used therapeutically for control of bladder spasms; subjects in the study group were being treated with this agent for clinical reasons and were therefore assigned to this group based solely on this criterion. All subjects were screened for a history of pulmonary disease or respiratory symptoms, recent viral syndrome or respiratory tract infection, and seasonal allergy. The Institutional Review Board for human studies of the Bronx Veterans Affairs Medical Center granted approval for the study. Informed consent of each subject was obtained prior to the investigation.

Spirometric measurements were obtained from subjects seated in their wheelchairs using an automated pulmonary function laboratory (SensorMedics System 2200, Yorba Linda, CA, USA). Baseline values of forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) were obtained for each subject according to the recommendations of the American Thoracic Society.[11] Spirometry results are expressed as absolute values and per cent predicted based upon the standards of Morris, *et al*.[12]

Subjects performed a series of tests, each involving five slow inhalations (inspiratory time 5 s) from functional residual capacity (FRC) to total lung capacity (TLC). All subjects were instructed not to hold their breath at TLC and to exhale slowly; the time between each inhalation was approximatley 10 s. Normal saline or increasing concentrations (0.025, 0.25, 2.5, 10 and 25 mg/ml) of histamine diphosphate (Freeman Industries, Tuckahoe, NY, USA) or methacholine (Provocholine, Roche Laboratories, Nutley, NJ, USA) were administered by a 8900 Salter disposable nebulizer (Asthmakit, Diemolding Healthcare Division, Canastota, NY, USA) containing 4 ml of solution and driven by air at a flow rate of 8 L/min with an output of 0.35 ml/min. Nebulization was performed by manual occlusion of a thumbport upon initiation of each breath. Spirometry parameters were measured 2–3 min after each set of five inhalations (saline and incremental concentrations of histamine or methacholine), or sooner if the subject experienced cough or chest tightness. The bronchial challenge test was terminated when either the FEV_1 decreased 20% or more from baseline (PC20) or the maximal concentration (25 mg/ml) was administered. The PC20 was calculated using a computer program which generated the value by linear interpolation from a logarithmic dose-response curve. A PC20 of less than 8 mg/ml was considered diagnostic for airway hyperreactivity.[13] An aerosolized β_2-agonist (2.5 ml of a 0.6% solution of metaproterenol sulfate) was immediately administered to all subjects exhibiting significant responses to either bronchoprovocative agent. For those subjects not responding to the maximal concentration of methacholine or histamine (25 mg/ml), a PC20 value of 25 was used in the calculation of mean PC20. The total cumulative units logarithmic concentration (mg/ml) of histamine or methacholine was calculated as the cumulative sum of number of breaths multiplied by concentration administered: 0.125, 1.375, 13.88, 63.88 and 188.88.

All data are expressed as means ± standard deviation and a natural logarithmic (ln) transformation was performed on PC20 values. An unpaired Student *t*-test was performed to assess differences between the

Table 1 Characteristics of methacholine groups

Group	*Age*	*DOI (year)*	*LOL*	*Smoking status*	*Medication*
Oxybutynin					
1	28	6	C-7, inc	never	valium
2	60	40	C-4, inc	never	none
3	49	22	C-6, com	former (1 year)	none
4	48	20	C-4, inc	never	none
5	35	1	C-6, com	former (5 years)	none
6	58	27	C-6, inc	current (13py)	valium
Mean ± s.d.	46 ± 12	19 ± 14			
Control					
1	63	39	C-6, inc	former (3 years)	none
2	63	22	C-7, inc	former (10 years)	none
3	44	19	C-7, inc	current (12py)	clonidine
4	49	27	C-6, com	current (33py)	none
5	54	18	C-6, inc	current (45py)	valium
6	26	1	C-5, com	current (12py)	valium
7	27	2	C-6, inc	current (8py)	none
Mean ± s.d.	47 ± 15	18 ± 13			

DOI = duration of injury, LOL = level of lesion, Inc = incomplete lesion, Com = complete lesion and py = pack years

oxybutynin and control groups in spirometry measures and the two different bronchoprovocations. In addition, a one-way ANOVA with *post-hoc* analysis was conducted using Scheffe's pairwise comparisons to determine whether there were differences among all four groups for mean lnPC20 values. The criterion for acceptance of statistical significance for all analyses was established at P less than 0.05.

Results

Age and duration of injury did not differ significantly between the oxybutynin and control groups (Tables 1 and 2). The study groups consisted primarily of subjects with C-4 to C-7 lesions who were never, former, or current smokers (Tables 1 and 2). Subjects in the oxybutynin groups had been receiving between 10 and 25 mg of the medication per day for 1–15 years. Baseline FVC, FEV_1 and per cent predicted values were reduced comparably in all four groups and mean FEV_1/FVC ratios did not differ significantly among the groups (Tables 3 and 4). There were no significant differences in spirometry parameters, level of lesion, duration of injury or smoking status (with the exception of the control/methacholine group which did not contain 'never' smokers) in responders *versus* non-responders. All 13 control subjects (both methacholine and histamine groups) and six oxybutynin subjects (entire histamine group) had a significant bronchoconstrictor response ($PC20 < 8$ mg/ml) (Tables 3 and 4). Mean lnPC20 values in the control methacholine group (0.40 ± 0.87 mg/ml), the control histamine group (1.14 ± 0.41 mg/ml), and the oxybutynin/histamine group (0.81 ± 1.1 mg/ml) did not differ significantly (Tables 3 and 4). The mean lnPC20 value for the oxybutynin/methacholine group was 3.2 mg/ml (Table 3).

Discussion

This study demonstrated that subjects with cervical SCI chronically maintained on oxybutynin chloride for control of bladder spasms exhibited a normal response to aerosolized methacholine. In contrast, bronchial responsiveness to aerosolized histamine was essentially identical to that found in subjects not receiving the agent. These findings parallel those obtained previously[8,14] in which subjects with cervical SCI chronically receiving baclofen, a GABA-B agonist administered chronically to control muscle spasms, did not demonstrate methacholine-associated bronchoconstriction, but did respond to histamine. Thus, findings that responsiveness to inhaled histamine was maintained in the presence of either oxybutynin or baclofen, agents with anticholinergic activities, suggest that histamine elicited bronchospasm by direct interaction with H_1 receptors on smooth muscle cells as opposed to stimulation of cholinergic or sensory nerves with secondary release of acetylcholine or neuropeptides. A number of animal studies have suggested that pulmonary effects of histamine are mediated in part through stimulation of the parasympathetic nervous system.[15,16] Moreover, some studies of asthmatic patients have shown that atropine or ipratropium bromide had no effect on histamine responses, while others have reported a protective effect.[17,18]

In the current study the specific mechanism by which oxybutynin inhibited methacholine responsiveness was not investigated. Our findings, however, that the bronchoconstrictor effects of methacholine were blocked while those of histamine were not suggest that oxybutynin acted primarily through anticholinergic pathways rather than causing generalized airway smooth muscle relaxation, which presumably would have resulted in a diminished histamine response.

Table 2 Characteristics of histamine groups

Group	*Age*	*DOI (year)*	*LOL*	*Smoking status*	*Medications*
Oxybutynin					
1	31	6	C-6, inc	current (11py)	valium
2	46	21	C-7, com	current (25py)	baclofen
3	33	9	C-7, com	never	none
4	23	3	C-6, inc	former (5 years)	valium
5	39	17	C-4, com	never	baclofen, valium
6	59	29	C-5, inc	current (25py)	baclofen
Mean ± s.d.	39 ± 13	14 ± 10			
Control					
1	33	10	C-6, inc	current (10py)	valium
2	47	5	C-7, inc	never	none
3	41	10	C-5, inc	never	baclofen
4	45	21	C-5, com	never	none
5	38	9	C-6, com	former (9 years)	dilantin, valium
6	24	4	C-6, com	current (6py)	baclofen, valium
Mean ± s.d.	38 ± 8	10 ± 6			

DOI = duration of injury, LOL = level of lesion, Inc = incomplete lesion, Com = complete lesion and py = packs years

Table 3 Baseline spirometry and PC20 results: methacholine groups

Group	*FVC*	*% Predicted*	*FEV1*	*% Predicted*	*FEV1/FVC*	*PC20 (mg/ml)*
Oxybutynin						
1	3.44	69	2.86	71	83	25
2	2.66	59	1.90	60	71	25
3	3.98	77	2.99	79	75	25
4	2.37	51	2.18	63	92	25
5	2.13	43	1.78	46	84	25
6	1.38	29	1.16	35	98	25
Mean ± s.d.	2.66 ± 0.93	55 ± 18	2.15 ± 0.69	59 ± 16	84 ± 10	25 (3.2)*
Control						
1	3.38	72	2.40	74	71	2.51
2	2.87	65	2.25	73	78	2.73
3	2.49	51	1.75	48	70	3.44
4	2.69	52	1.89	50	70	1.70
5	2.28	51	1.86	57	82	2.34
6	2.12	40	1.83	43	86	0.31
7	3.00	66	2.58	69	86	1.40
Mean ± s.d.	2.70 ± 0.20	57 ± 11	2.10 ± 0.10	59 ± 13	78 ± 8	2.0 ± 1.1 (0.4 ± 0.87)*

All lung function parameters are expressed in liters and percentage predicted. FVC = forced vital capacity, FEV1 = forced expiratory volume in 1 s, PC20 = concentration of provocative agent causing a decrease in FEV1 by 20 per cent. * = PC20 means ± s.d. presented as logarithmic values

Table 4 Baseline spirometry and PC20 results: histamine groups

Group	*FVC*	*% Predicted*	*FEV1*	*% Predicted*	*FEV1/FVC*	*PC20 (mg/ml)*
Oxybutynin						
1	2.26	46	2.00	51	88	4.91
2	2.44	51	1.67	46	68	0.52
3	3.76	69	3.33	79	89	4.59
4	4.05	69	3.31	72	82	6.35
5	1.89	32	1.57	36	83	0.92
6	2.01	43	1.56	47	78	1.35
Mean ± s.d.	2.74 ± 1.06	52 ± 16	2.17 ± 0.74	55 ± 14	81 ± 9	3.4 ± 2.9 (0.8 ± 1.1)*
Control						
1	3.45	63	3.10	74	90	3.72
2	4.93	87	3.56	86	72	1.71
3	3.64	66	2.77	67	76	5.95
4	3.02	56	2.67	66	88	3.08
5	2.20	41	1.97	49	90	0.74
6	2.03	38	1.77	41	88	2.11
Mean ± s.d.	3.21 ± 1.06	59 ± 18	2.64 ± 0.68	64 ± 16	84 ± 7.9	3.4 ± 1.4 (1.1 ± 0.41)*

All lung function parameters are expressed in liters and percentage predicted. FVC = forced vital capacity, FEV1 = forced expiratory volume in 1 s, PC20 = concentration of provocative agent causing a decrease in FEV1 by 20 per cent. * = PC20 means ± s.d. presented as logarithmic values

Similarly, findings in guinea pigs that oxybutynin pretreatment attenuated carbachol-induced collapse in conscious animals and inhibited methacholine-induced bronchoconstriction in anesthetized animals suggest that these effects were also mediated through anticholinergic activity.[5] By comparison, a number of *in vivo* and *in vitro* animal studies involving the gastrointestinal system, uterus and urinary bladder have demonstrated that oxybutynin acts primarily through direct potent musculotropic spasmolytic actions, with mild anticholinergic and antihistaminic properties.[1,3] Additional studies have suggested that the direct relaxant effect is mediated through local anesthetic activity caused by displacement of membrane-bound Ca^{2+}, inhibition of excitation contraction coupling, and disruption of transmembrane Ca^{2+} fluxes.[3]

The design of this study was based on clinical criteria. Since the oxybutynin study group were being treated for urinary complications it was not feasible to implement a cross-over design. Furthermore, oxybutynin can produce unpleasant side effects and should not be prescribed unless clinically warranted; thus, we

felt that randomly prescribing oxybutynin had the potential to cause significant consequences that outweighed the possibility of biased results due to our pre-selection of subjects. In addition, although the control group receiving aerosolized methacholine did not include subjects who were never smokers, we have previously reported[19] that smoking status is not a contributing factor to airway hyperreactivity in subjects with cervical SCI.

This study further illustrates that subjects with cervical SCI not receiving oxybutynin or baclofen therapy demonstrate airway hyperresponsiveness following inhalation of the pharmacological agents histamine or methacholine. Recently we have shown that these subjects are also hyperresponsive to physicochemical agents, such distilled water.[10] Findings of hyperresponsiveness to two provocative agents with divergent mechanisms of action in addition to a hypo-osmolar agent suggest that hyperreactivity is a reflection of non-specific bronchial responsiveness, also a common feature of patients with asthma, who generally exhibit hypersensitivity to an array of stimuli including physical, physicalchemical, chemical, and pharmacological agents.[20]

Tentative unifying explanations for airway hyperreactivity in this population may include autonomic nervous system imbalance or altered mechanical properties of the lungs. Previously we proposed that methacholine-associated airway hyperreactivity in subjects with cervical SCI was due to loss of sympathetic nervous system innervation of the lungs, which originates from thoracic vertebrae one through six, thereby leaving intact unopposed parasympathetic bronchoconstrictor activity.[8] Similarly, it has been proposed that some features of asthma represent amplified cholinergic activity.[20] Although the parasympathetic nervous system is the dominant neural bronchoconstrictive system in humans, there is no evidence that cholinergic tone is increased in asthma, suggesting that cholinergic hyperresponsiveness is unlikely to be a primary abnormality of asthma.[21] Alternatively, it has been proposed that airway hyperreactivity in asthma represents an intrinsic impairment of stretch of airway smooth muscle that normally occurs with deep inspiration.[22,23] Since lung volumes are decreased and deep inhalation is not possible in subjects with cervical SCI due to respiratory muscle paralysis, it is conceivable that hyperresponsiveness is due to inadequate stretch of airways with deep breathing. Continued investigation into this unique model of airway hyperreactivity may help to further uncover relationships between airway hyperresponsivness and factors that lead to increased airway narrowing.

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