CLINICAL RESEARCH

Bronchial hyperreactivity in response to inhalation of ultrasonically nebulised solutions of distilled water and saline

ROBIN E SCHOEFFEL, SANDRA D ANDERSON, ROGER E C ALTOUNYAN

Abstract

To assess non-specific bronchial reactivity the effect of inhaling ultrasonically nebulised solutions of distilled water and hypotonic (0·3%), isotonic (0·9%), and hypertonic (2·7%, 3·6%) saline was investigated in 10 asthmatic patients and nine normal subjects. Expired ventilation and the maximum percentage fall in forced expiratory volume in one second (FEV_1) were recorded. The sensitivity to the inhaled solutions was determined by measuring the ventilation required to induce a fall in FEV_1 of 20% from the prechallenge value. Hypotonic and hypertonic but not isotonic solutions caused a significant fall in FEV_1 in the asthmatic subjects. Normal subjects showed no response to either distilled water or 3·6% saline, the only solutions with which they were challenged.

The method used for this challenge is rapid, simple, and inexpensive and provides a new means of diagnosing non-immunologically mediated bronchial hyperreactivity.

Introduction

Bronchial hyperreactivity in response to non-immunological stimuli including methacholine, histamine, exercise, and cold air has been well documented.[1-3] Allegra and Bianco[4] reported that in patients with asthma a significant increase in airways resistance occurred after the inhalation of ultrasonically nebulised distilled water. To determine whether a change in the tonicity of the inhaled solution might vary the airway response we investigated a group of asthmatic patients and normal subjects who inhaled ultrasonically nebulised solutions of saline that varied in concentration up to 3·6%.

Department of Thoracic Medicine, Royal Prince Alfred Hospital, Camperdown, New South Wales 2050, Australia

ROBIN E SCHOEFFEL, SRN, research assistant
SANDRA D ANDERSON, PHD, principal scientific officer
ROGER E C ALTOUNYAN, MB, visiting clinical pharmacologist (Fisons Ltd, Loughborough, England)

Subjects and methods

We studied 10 patients aged 16–55 years with clinically recognised asthma who were taking a beta-sympathomimetic aerosol regularly for control of their symptoms. Nine non-asthmatic subjects volunteered to serve as controls.

Forced expiratory volume in one second (FEV_1) was measured (Minato Autospirometer, Osaka, Japan) in the patients before and after the inhalation of ultrasonically nebulised distilled water and four concentrations (0·3%, 0·9%, 2·7%, 3·6%) of saline. A $MistO_2gen$-Electronic Nebulizer (California, USA) was used in all studies. Water was always given for the first challenge, while the subsequent challenges with saline were performed in random order. The challenges were carried out on five separate days at the same time of day, at least four hours but in most cases more than six hours after medication. Two inhalational challenges were performed in the nine controls, one with water and the other with 3·6% saline. The protocol was approved by the ethics committee of the hospital and informed consent obtained.

The subjects inhaled the solution through a two-way valve and the expired air passed through a Dräger Volumeter. Before each challenge FEV_1 was measured before and after the subject inspired 40 l of room air. Two minutes later the challenge with the ultrasonically nebulised solutions began.

Initially 5 or 10 l of the solution was inhaled and 30 seconds later three or four measurements of FEV_1 made. If after challenge the FEV_1 had fallen by 10% or more from the value measured immediately before challenge a further 5 or 10 l of the solution was inhaled two minutes later and the measurement of FEV_1 repeated. The volume inhaled in each subsequent test period varied according to the change in FEV_1 after the previous test. If the reduction in FEV_1 after the initial test was less than 10% the volumes used in subsequent tests were 20 l, 40 l, 80 l, 80 l, and 80 l, until a fall in FEV_1 of at least 20% from the prechallenge level was observed or 310 l had been inhaled. The occurrence of coughing during each challenge was recorded.

Bronchial reactivity to the inhaled solutions was assessed by determining the total ventilation required to induce a fall in FEV_1 of 20%. This value was obtained by plotting, on semi-log paper, the fall in FEV_1 (expressed as a percentage of the prechallenge value) after each test against the cumulative ventilation required to induce that change in FEV_1.

In the patients an inhalational challenge with histamine diphosphate was performed using the standardised technique described by Chai *et al.*[5] The dose of histamine required to induce a fall in FEV_1 of 20% is expressed in dose units. The histamine challenge was performed 30 minutes after the challenge with 0·9% saline unless a fall in FEV,

greater than 10% had been recorded after that challenge, in which case it was performed on a separate day.

To assess the reproducibility of the response to ultrasonically nebulised water two challenge studies were performed on separate days in eight of the patients.

A two-way analysis of variance was used to determine whether there was a difference between tests. Duncan's multiple range test was used to determine the level of significance.[6]

Results

Table I shows the total ventilation required to induce a fall in FEV_1 of 20% for each solution in each patient and the dose of histamine required to induce the same fall in FEV_1. Table II shows the significance of differences between the tests. Table III shows the mean maximum percentage falls in FEV_1 observed after the challenges and the mean ventilation required to induce these falls in FEV_1. There was no significant difference in the maximum percentage falls in FEV_1 observed after inhalation of distilled water (40·3 ± SEM 4·2%) and 3·6% saline (36·5 ± 3·3%), and the ventilations were not significantly different (94·0 ± 33·4 l and 91·0 ± 23·9 l, respectively). Four patients showed a fall in FEV_1 greater than 20% after inhaling 0·9% saline. The bronchoconstriction induced by the solutions was rapidly reversed by salbutamol.

All the normal subjects inhaled 310 l of each solution without registering a fall in FEV_1 of 20%.

The prechallenge values of FEV_1 on each day were similar for each patient. The within-patient coefficient of variation for the resting FEV_1 was 11·1%. A small fall in FEV_1 occurred in response to breathing 40 l of room air before challenge (mean 4·9 + SD 6·2%). The response to challenge with water was reproducible. The mean difference in the total ventilation required to induce a 20% fall in FEV_1 between the two tests was 9·3 + SD 7·6 l.

Four patients coughed in response to inhalation of distilled water and three, two, seven, and five in response to inhalation of 0·3%, 0·9%, 2·7%, and 3·6% saline, respectively.

TABLE I—*Ventilation required (l) to induce fall in FEV_1 of 20% of prechallenge value in 10 asthmatic patients who inhaled distilled water and saline, and dose of histamine required to induce same fall in FEV_1*

Case No	Distilled water	Saline				Histamine (IDU*)
		0·3%	0·9%	2·7%	3·6%	
1	27·0	>250·0	>310·0	37·5	39·0	0·23
2	30·5	5·5	21·0	110·0	28·5	0·44
3	180·0	250·0	>310·0	>220·0	230·0	27·0
4	11·0	21·5	260·0	275·0	46·0	2·2
5	3·9	3·2	>310·0	>310·0	87·0	21·0
6	32·0	>310·0	>310·0	140·0	53·0	27·0
7	1·3	300·0	>310·0	23·0	8·5	2·05
8	38·0	39·0	>310·0	200·0	12·5	3·3
9	14·0	12·5	74·0	41·0	4·5	0·15
10	13·2	8·5	>310·0	>310·0	16·5	0·56

*IDU = Inhalation dose unit = 1 g histamine/l.

TABLE II—*Significance of differences in responses to inhaled solutions of water and saline in asthmatic patients as measured by ventilation and change in FEV_1*

	Total ventilation to induce 20% fall in FEV_1	Maximum percentage fall in FEV_1	Total ventilation
0·9% *v* water	<0·001	<0·001	<0·001
0·9% *v* 0·3%	<0·01	<0·01	<0·01
0·9% *v* 2·7%	NS	<0·001	NS
0·9% *v* 3·6%	<0·001	<0·001	<0·001
Water *v* 0·3%	NS	NS	NS
Water *v* 2·7%	<0·01	<0·05	<0·05
Water *v* 3·6%	NS	NS	NS
3·6% *v* 0·3%	NS	NS	NS
3·6% *v* 2·7%	<0·05	NS	<0·05
0·3% *v* 2·7%	NS	NS	NS

TABLE III—*Mean (± SEM) maximum fall in FEV_1 and total ventilation required to induce that fall after inhalation of distilled water and saline solutions in 10 asthmatic patients*

	Distilled water	Saline			
		0·3%	0·9%	2·7%	3·6%
Maximum fall in FEV_1 (%)	10·3 ± 4·2	33·1 ± 5·6	12·5 ± 4·1	27·2 ± 3·6	36·5 ± 3·3
Total ventilation (l)	94·0 ± 33·4	144·5 ± 43·2	288·0 ± 22·6	201·0 ± 34·2	91·0 ± 23·9

Discussion

These results confirm the observations of others that distilled water is a potent stimulus for inducing an increase in airway resistance in patients with asthma but not in normal subjects.[4 7] This study extends these observations and indicates that the osmolarity of the solution may be an important determinant of the response. Both hypotonic and hypertonic solutions of saline were more potent in provoking a 20% fall in FEV_1 than isotonic solutions of saline. We are unaware of any previous reports of bronchial hyperreactivity in response to inhalation of solutions of hypertonic saline.

A fall in FEV_1 of 20% or more in response to inhalation of 3·6% saline and distilled water was observed only in the patients with asthma, indicating that this non-immunological stimulus for provoking bronchoconstriction may be useful in distinguishing patients with hyperreactive airways. All the patients tested were sensitive to inhaled histamine and had documented exercise-induced asthma, both of which are non-immunological stimuli of bronchial hyperreactivity. The technique used for measuring bronchial hyperreactivity in this study is simple and does not require expensive equipment. The temperature of the inhaled solution is not critical and may vary from 22°C to 35°C without changing the response. Extreme caution must be taken to ensure that the inhaled solutions are sterile. The time taken to perform the challenge with water or 3·6% saline is relatively short compared with exercise or histamine challenge. The bronchoconstriction induced is readily reversible with salbutamol.

In one patient, in whom challenges were repeated while she was suffering from a respiratory tract infection, the response to both water and 3·6% saline was absent. The importance of this observation is not known.

In a separate study using a similar protocol we found that inhalation of 20% dextrose induces changes in FEV_1 similar to those obtained in this study with distilled water and 3·6% saline. Thus the osmolarity of the solution appears to be the important determinant of the airway response. The mechanism by which a change in osmolarity induces a reduction in FEV_1 in patients with asthma is not clear, though there are several possibilities. The lung irritant receptors may be stimulated directly by a change in the osmolarity of the fluid lining the respiratory tract. Furthermore, histamine may be released locally from mast cells lying superficially in the bronchial mucosa. A change in osmotic pressure around a mast cell may induce movement of water into the cell in the case of hypotonic solutions or out of the cell in the case of hypertonic solutions. Mast cells release histamine in hypotonic solutions,[8] and basophils release histamine in response to hypertonic solutions.[9] The release of histamine may induce contraction of smooth muscle by acting either directly on the smooth muscle or reflexly by the vagus nerve.

Since the particles generated by the Mistogen nebuliser are small deposition in the lung is probably diffuse. Other measurements of lung function are required to determine whether the peripheral airways become obstructed before a fall in FEV_1 occurs. The asthma experienced by some patients in winter may perhaps be due not only to cooling of the airways but also to the inhalation of fine particles of water as occur in fog.

Thus we found that inhalation of ultrasonically nebulised solutions of distilled water and hypotonic and hypertonic but not isotonic saline induced a significant reduction in FEV_1 in asthmatic patients. This bronchial hyperreactivity was specific to the patients and no response was observed in normal subjects. The method used for the challenge is rapid, simple, and

inexpensive and provides a new technique for the diagnosis of non-immunologically mediated bronchial hyperreactivity.

This work was supported by a grant from the Asthma Foundation of New South Wales.

Requests for reprints should be sent to SDA.

References

[1] Curry JJ. Comparative action of acetyl-beta methylcholine and histamine on the respiratory tract in normals, patients with hay fever and subjects with bronchial asthma. *J Clin Invest* 1947;**26**:430-8.

[2] Anderson SD, Silverman M, Godfrey S, Konig P. Exercise-induced asthma; a review. *Br J Dis Chest* 1975;**69**:1-39.

[3] Deal EC, McFadden ER, Ingram RH, Breslin FJ, Jaeger JJ. Airway responsiveness to cold air and hyperpnea in normal subjects and in those with hay fever and asthma. *Am Rev Resp Dis* 1980;**121**:621-8.

[4] Allegra L, Bianco S. Non-specific broncho-reactivity obtained with an ultrasonic aerosol of distilled water. *Eur J Respir Dis* 1980;**61**, suppl 106: 41-9.

[5] Chai H, Farr RS, Froehlich LA, *et al.* Standardisation of bronchial inhalation challenge procedures. *J Allergy Clin Immunol* 1975;**56**:323-7.

[6] Duncan DB. Multiple range and multiple F-tests. *Biometrics* 1955;**11**:1-42.

[7] Cheney FW, Butler J. The effects of ultrasonically-produced aerosols on airway resistance in man. *Anesthesiology* 1968;**29**:1099-106.

[8] Kaliner M, Austen KF. Cyclic AMP, ATP and reversed anaphylactic histamine release from rat mast cells. *J Immunol* 1974;**112**:664-74.

[9] Findlay SR, Lichtenstein LM. Basophil "releasability" in patients with asthma. *Am Rev Respir Dis* 1980;**122**:53-9.

(*Accepted* 7 *September* 1981)

DR antigens and rheumatoid arthritis: a study of two populations

J C WOODROW, F E NICHOL, G ZAPHIROPOULOS

Abstract

The prevalence of HLA-DR antigens was determined in a group of white patients with rheumatoid arthritis, similar patients originating from the Indian subcontinent, and corresponding controls. Rheumatoid arthritis was found to be highly associated with DR4 in the white patients but with DR1 in the Indian patients.

These results raise the possibility that the DR antigens themselves do not play a part in increasing susceptibility to rheumatoid arthritis, but the locus for increased susceptibility is probably closely linked to the DR locus.

Introduction

An association has been shown between HLA-DR4 and rheumatoid arthritis.[1-3] The populations studied have been largely European or of European origin. When an HLA antigen is shown to be positively associated with a disease two main possibilities arise. Firstly, the HLA antigen itself may play a direct part in producing increased susceptibility. Secondly, the association may arise because the susceptibility gene is not that determining the HLA antigen itself but is present at a locus within the HLA region and is in linkage disequilibrium with the gene for the HLA antigen. Supportive evidence for this second concept is afforded when what appears to be the same disease is associated with different HLA antigens in different ethnic populations. To elucidate this further with regard to rheumatoid arthritis we tested for their DR phenotypes two ethnically different groups of patients and corresponding healthy controls.

University Department of Medicine, Royal Liverpool Hospital, Liverpool L69 3BX
J C WOODROW, MD, FRCP, professor

Leicester Royal Infirmary, Leicester
F E NICHOL, MD, MRCP, senior registrar in rheumatology

Coventry and Warwickshire Hospital, Coventry CV1 4FH
G ZAPHIROPOULOS, MRCP, consultant rheumatologist

Patients and methods

Two separate studies were carried out and the results analysed together. In the Liverpool study DR typing was carried out in 100 consecutive white patients with seropositive erosive rheumatoid arthritis attending a rheumatology clinic. Typing was also carried out on blood samples from 100 healthy subjects (medical, nursing, and laboratory staff) living in the same geographical area.

In the Midlands study DR typing was similarly carried out in 35 patients originating from the Indian subcontinent and having seropositive rheumatoid arthritis. Typing was also performed in 42 healthy controls of the same ethnic origin living in the same geographical area.

DR typing was done in one laboratory (department of medicine, Liverpool University) by means of a cytotoxicity technique using an adaptation of the two-colour fluorescence method.[4] The panel of sera used permitted typing for seven DR specificities. The relative risks were calculated using Haldane's modification of the Woolf method.[5] The derived probability values (p) are twice the p values obtained from the normal probability integral, giving values almost identical with those obtained with Fisher's exact test. Taking into account that seven antigens were tested for, a significant association was associated with a p value of 0·007 or less.

Results

Table I shows the numbers of patients and controls positive for each DR antigen in the two series. In the Liverpool study there was a highly significant positive association with DR4, the relative risk of a person positive for DR4 getting rheumatoid arthritis being 4·56 ($p = 0{\cdot}61 \times 10^{-6}$). There was a non-significant increase in the relative risks associated with DR1 and DR3. The relative risks for DR6 and DR7 were 0·22 and 0·37 respectively, and the corresponding p values of 0·005 and 0·0067 suggest that the negative associations were real. In the Midlands study a significant positive association was found with DR1, with a relative risk of 7·0 ($p = 0{\cdot}0022$). There was a small non-significant increase in the prevalence of DR4. No significant negative associations were found.

Table II gives the outcome of statistical analysis of the results in the two series. There was significant heterogeneity between the two populations in respect of DR1 ($p = 0{\cdot}013$) and DR4 ($p = 0{\cdot}049$).

Discussion

The main finding in this study was the significantly different patterns of association of DR antigens with rheumatoid