Ascorbic acid in bronchial asthma

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Summary

Sixteen White children with bronchial asthma were divided into two groups; one received standard anti-asthma chemoprophylaxis (SAC) and the other SAC supplemented with 1 g ascorbic acid (Redoxon) given as a single daily dose for a 6-month period. In 10 patients the effects of ascorbic acid on exercise-induced bronchoconstriction (EIB) were assessed by comparing the pre-ascorbic acid results with those obtained $2^1/_2$ hours after the intravenous injection of 1 g ascorbic acid. Immunological investigations performed on the two groups were assessment of polymorphonuclear leucocyte (PMNL) motility, phagocytosis and nitroblue tetrazolium reduction and measurement of secretory IgA, serum immunoglobulin and total haemolytic complement levels and levels of the components C3 and C4, α_1-antitrypsin, antistreptolysin O (ASO), C-reactive protein and antibodies to certain respiratory viruses. These investigations were performed before and 1, 3 and 6 months after the commencement of therapy. Radio-allergosorbent testing for sensitivity to four common allergens was carried out at the outset and after 6 months of therapy. Injection of ascorbic acid had no detectable effects on the degree of EIB. Slight but not significant immunological changes were observed in the SAC group over the 6-month study period. However, in the SAC plus ascorbic acid group significantly improved PMNL motility and decreased ASO levels and reduced (although not to a significant extent) IgE levels and titres of antibodies to the respiratory viruses were observed.

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In a previous study[1] we observed that in a group of 10 children receiving standard anti-asthma chemoprophylaxis (SAC) supplemented with 1 g ascorbic acid daily both humoral and cellular immune reactivity improved. However, since no control group was included (i.e. on SAC only) the improvements in immunological function could not be attributed to SAC or ascorbic acid alone or to a synergistic effect of both. In this investigation we have assessed the effects of SAC only and SAC plus ascorbic acid on certain cellular and humoral immune functions in a group of children with bronchial asthma.

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Patients and methods

Twelve boys and 4 girls (average age 9,5 years) were investigated. Informed parental consent was obtained in all cases. The children had all been referred to the Paediatric Respiratory Clinic at the H. F. Verwoerd Hospital, Pretoria, for investigation and management of asthma which had been difficult to control. Patients were randomly selected on the basis of a history of recurrent respiratory infections and asthma confirmed by lung function studies.

All the patients had perennial asthma. Ten (7 boys and 3 girls) had moderate asthma with an average resting maximal mid-expiratory flow rate (MMEFR) of 66% of normal. Six (5 boys and 1 girl) had more severe asthma with an average MMEFR of 36% of normal.

Patients were graded according to the history and the treatment required to keep them in reasonable health. This correlated well with the lung function findings.

All patients received 3 daily doses of sodium cromoglycate (Lomudal) and either fenoterol (Berotec) or salbutamol (Ventolin). Those with more severe asthma also received daily aminophylline. Patients were randomly assigned to two groups. Group 1 (4 moderate and 3 severe asthmatics) received 1 g ascorbic acid (Redoxon) as a single dose each morning in addition to the standard therapy. Group 2 (6 moderate and 3 severe asthmatics) received only standard therapy (Table I). No patients received glucocorticoids before or during the study and none required hospitalization for asthma during the course of the trial. Stool examinations revealed no parasites. During the trial none of the patients moved to a different environment and no change in allergen avoidance procedures was instituted.

Lung function testing

All the children had a typically obstructive resting flow volume curve with an MMEFR of less than 75% of the expected normal value (average value 53%). All had exercise-induced bronchoconstriction on treadmill testing performed according to the standard method described by Silverman and Anderson.[2] A fall in the peak expiratory flow rate (PEFR) and an MMEFR of more than 15% of the resting value was considered significant. Our patients had an average fall in MMEFR to 46% of the resting values.

The effect of ascorbic acid on exercise-induced bronchoconstriction was studied in the first 10 patients. An exercise study was performed at 08h00. Ascorbic acid (1 g) was then administered intravenously, and the patient rested for $2^1/_2$ hours. The exercise study was then repeated and compared with the original study. Two patients improved slightly, 2 deteriorated slightly and in 6 the differences between the two studies were not significant. No beneficial or detrimental effect of ascorbic acid on exercise bronchoconstriction could be demonstrated.

Polymorphonuclear leucocyte (PMNL) functions

PMNLs were obtained from heparinized venous blood and resuspended in Hanks's balanced salt solution as previously described.[1] Investigations of PMNL motility, phagocytosis and nitroblue tetrazolium (NBT) reduction were performed using

TABLE I. SUMMARY OF THE CLINICAL AND IMMUNOLOGICAL FINDINGS ON INITIAL INVESTIGATION

Patient No.*	Age (yrs)	Sex	Growth percentile		Clinical grading	MMEFR (% of normal)	Total serum IgE level (IU/ml)	RAST sensitivity to				Immunological abnormalities
			Weight	Height				Grass	Mite	House dust	Cat	
1	8	M	25	25	Moderate	50	112	2	3	3	3	↑ASO; ↑VA
2	12	M	3	25	Severe	57	270	1	—	—	—	↑VA
3	8	M	3	3	Moderate	70	44	—	—	—	—	—
4	8	F	50	45	Moderate	60	700	3	1	2	4	↑VA
5	10	M	40	50	Moderate	55	170	—	—	—	3	↑ASO; ↑VA
6	6	F	—	—	Moderate	65	355	—	—	—	—	↑ASO
7	10	F	25	3	Severe	30	810	2	2	2	4	—
8	10	M	75	50	Severe	33	175	—	—	—	—	↓CTX
9	11	F	50	50	Moderate	80	310	1	—	—	—	—
10	12	M	10	10	Moderate	62	250	1	—	2	—	—
11	6	M	25	25	Moderate	74	480	1	—	—	—	↓CTX; ↑ASO; ↑VA
12	9	M	10	25	Moderate	51	310	4	—	—	—	↑ASO; ↑VA
13	8	M	25	45	Moderate	78	580	2	2	3	3	—
14	9	M	25	50	Severe	30	49	—	—	—	1	↓CTX; ↑ASO; ↑VA
15	13	M	50	50	Severe	44	290	—	—	—	—	↑ASO
16	8	M	25	50	Severe	25	210	4	—	2	—	↑VA

*Patients 1 - 9 received SAC only and patients 10 - 16 SAC plus ascorbate.
↑ASO = elevated titres of antibodies to ASO; ↑VA = elevated titres of antibodies to respiratory viruses; ↓CTX = depressed PMNL motility.

well-documented techniques and the results of motility studies expressed as PMNLs per high-power microscopic field (cells/HPF).

Serological tests

Serum IgG, IgA (as well as secretory IgA) and IgM levels, total haemolytic complement activity and levels of C3 and C4, C-reactive protein (CRP), α_1-antitrypsin (AAT) and antibodies to streptolysin O (ASO) were quantitated as previously described.[1] Serum IgE levels were quantitated by the Phadebas IgE PRIST test (Pharmacia) and values of $\geq$ 100 IU/ml were considered to be elevated. Antibodies to the respiratory viruses influenza A, B and C para-influenza 1, 2 and 3, respiratory syncytial virus and adenovirus as well as to *Mycoplasma pneumoniae* were measured using a complement fixation test. Levels of specific IgE against the common allergens *Cynodon dactylon* (grass), *Dermatophagoides pteronyssimus* (mite), house dust and cat epithelium were measured by the radio-allergosorbent test (RAST) and results expressed according to extent of positivity on the scale 0-4.

Serum ascorbate levels were measured by the 2,4-dinitrophenylhydrazine method.[3]

These tests were performed before therapy and 1, 3 and 6 months thereafter, with the exception of the RAST, which was performed at the outset and at 6 months only. Only the clinician (I.H.) was aware of which therapeutic regimen each of the patients was receiving.

Results

Calculation and expression of results

Results are expressed as mean ± SE for each investigation for both groups. Statistical analyses were performed by Student's *t* test (paired *t* statistic).

Initial clinical and laboratory findings (Table I)

Patients 1-9 received SAC only and patients 10-16 SAC plus ascorbic acid. Of the 9 patients in the SAC group, 1 had low PMNL motility, 3 elevated levels of ASO and 4 elevated titres of antibodies to the respiratory viruses. Of the 7 who received SAC plus ascorbic acid, 2 had reduced PMNL motility and 4 elevated ASO levels; tests for antibodies to the respiratory viruses were positive in 4.

Effects of therapy on PMNL functions

No abnormalities of PMNL phagocytosis of *Candida albicans* or of spontaneous and stimulated NBT reduction were observed in any patient on initial investigation, and these findings were not affected by either of the regimens (results not shown). PMNL motility showed a slight improvement in the SAC group, and in the patient with reduced migration motility returned to normal. Significant sustained stimulation of PMNL motility was observed in the group receiving SAC plus ascorbic acid. These results with autologous endotoxin-activated serum as the leuco-attractant are shown in Table II.

Effects of therapy on serological components

Insignificant decreases in serum levels of IgG, IgA and IgM and secretory IgA were observed in both groups. Levels of total haemolytic complement, C3, C4 and AAT were normal at the outset and remained unchanged in both groups. CRP was not detectable in the serum of any patient at any time (results not

TABLE II. EFFECTS OF SAC WITH AND WITHOUT ASCORBIC ACID ON PMNL MIGRATION TO EAS*

Time of testing	SAC only	SAC + ascorbic acid
Before therapy	**215 ± 18**	**229 ± 29**
After 1 mo.	**267 ± 46**	**293 ± 19†**
After 3 mo.	**185 ± 27**	**265 ± 31**
After 6 mo.	**230 ± 20**	**303 ± 44†**

*Results expressed as cells/HPF (mean ± SE).
†$P < 0,05$.

shown). The effects of therapy on serum levels of IgE and ASO are shown in Table III. Treatment with SAC and ascorbic acid was associated with a significant reduction in ASO levels and insignificant decreases in IgE levels. SAC alone was associated with reduced ASO levels and slightly increased IgE levels, but these changes were not significant. In the SAC and SAC plus ascorbic acid groups there were totals of 17 and 15 positive titres to the various respiratory viruses, which decreased to 12 and 9 respectively after 6 months of therapy, representing decreases of 30% and 40% respectively.

Serum ascorbic acid levels

Intravenous injection of 1 g ascorbic acid elevated the serum ascorbic acid concentrations to > 40 μg/ml $2^1/_2$ hours after injection. Ingestion of ascorbic acid increased the mean serum concentration from 10,8 ± 1,3 μg/ml to 24,1 ± 2,4 μg/ml.

Patients

No patient in either group experienced a severe attack of asthma during the investigation or had an episode of serious lower respiratory infection. All patients in both groups (with the exception of patient 7, who required inhalant corticosteroids to achieve adequate control) showed improved lung function as determined on optimal therapy by a return to normal PEFR and a rise in MMEFR to greater than 85% of the expected normal value. There were no obvious differences between the two groups.

Discussion

It has been reported that in adult volunteers bronchoconstriction caused by inhalation of histamine aerosols[4] and textile dusts[5] was significantly reduced by prior administration of 0,5 - 1 g ascorbic acid and that this may relate to the antihistaminic activity of the vitamin.[6] However, other investigators were unable to detect any protective effects of ascorbic acid on the intensity of allergen- or histamine-induced bronchoconstriction in mild asthmatics.[7] Recently Anah *et al.*[8] have reported that ingestion of ascorbic acid by children with bronchial asthma in whom the attacks were precipitated by virus infections decreased the frequency and severity of asthmatic episodes. The trial was performed on a double-blind basis over a 14-week period. Withdrawal of ascorbic acid was associated with an increase in the frequency of attacks.

We have previously reported that SAC supplemented with 1 g ascorbic acid daily for 6 months in a group of children with bronchial asthma was associated with improved lung function and immune reactivity.[1] However, the study was uncontrolled and definite conclusions could not be drawn. In the present study we have shown that ascorbic acid *per se* has no protective effect against exercise-induced bronchoconstriction. This was shown in the group of 10 patients who received intravenous injections of ascorbic acid to obtain high serum ascorbic acid levels and were then tested for exercise-induced bronchospasm, and also by comparing the effects of SAC with and without ascorbic acid over an extended period.

However, significant differences were observed between the two groups when PMNL motility, ASO levels and serum IgE levels were investigated. Ascorbic acid supplementation was associated with significantly improved PMNL motility and decreased ASO levels. There was also a drop in serum IgE levels, but this was not significant. Improvements (although not significant) were noted in PMNL motility and ASO levels in the control group (no ascorbic acid), indicating that the effects in the group receiving SAC plus ascorbic acid are probably synergistic. Obviously a third group of patients receiving ascorbate only (i.e. no SAC) would have been of interest. However, in view of our findings that ascorbic acid alone conferred no protection against exercise-induced bronchoconstriction the inclusion of such a group could not be considered.

In view of these findings it is our opinion that ascorbic acid may be a useful supplement to SAC in some patients with bronchial asthma to confer a measure of protection against the acquired transient defects of PMNL migration and lymphocyte responsiveness to antigens which are often associated with elevated IgE levels.[9] The raised ASO levels observed in patients with bronchial asthma confirm our previous observation of this association.[1] However, it is not yet known whether infections with *Streptococcus pyogenes* are the cause or the consequence (as a result of atopy-associated decreased immunity) of some cases of bronchial asthma. The high incidence of positive antibody titres to the respiratory viruses also indicates decreased immunity, which may be primary (as a result of production of virus-specific IgE[10]) or secondary. Interestingly, the two regimens produced a similar drop in these titres, indicating that SAC has an enhancing effect on local immunity to the respiratory viruses.

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TABLE III. THE EFFECTS OF SAC WITH AND WITHOUT ASCORBIC ACID ON SERUM LEVELS OF IgE* AND ASO†

	SAC only		SAC + ascorbic acid	
Time of testing	IgE	ASO	IgE	ASO
Before ascorbate	**327 ± 87**	**140 ± 43**	**310 ± 66**	**259 ± 67**
After 1 mo.	**242 ± 60**	**109 ± 25**	**246 ± 51**	**164 ± 39‡**
After 3 mo.	**523 ± 167**	**94 ± 31**	**211 ± 38**	**Not done**
After 6 mo.	**396 ± 141**	**94 ± 18**	**253 ± 60**	**127 ± 44‡**

*Results expressed as IU/ml serum (mean ± SE).
† Results expressed as Todd units (mean ± SE).
‡$P < 0,025$.

measuring antiviral antibody titres and serum immunoglobulin and α_1-antitrypsin levels respectively.

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