Evaluation of Complement (C3) Inhibition
_In Vitro_ by Drugs Used in the Management of Bronchial Asthma*

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The complement system is an integral part of the body's defence mechanism.\(^1\) Complement activation has been proposed as having a key role to play in the pathogenesis of various diseases including rheumatoid arthritis, systemic lupus erythematosus\(^3,4\) and recently bronchial asthma.\(^5-8\)

In a previous report, we demonstrated that common inhalant allergen, housedust mite, *Dermatophagoides farinae* activates the complement system _in vitro_ by classical as well as the alternative pathways in bronchial asthma patients, thus indicating that the complement system may play an important role in the pathogenesis of bronchial asthma.\(^8\) The anaphylatoxins C3a and C5a formed as a result of complement activation by the allergen may thus lead to inflammatory changes as well as bronchospasm. The present study was undertaken to evaluate whether the drugs used in the management of bronchial asthma, namely, disodium cromoglycate, hydrocortisone, salbutamol, aminophylline and diethylcarbamazine citrate (Hetrzan\(^\text{®}\)) can modulate the activity of the complement system _in vitro_. Although the mechanisms of some of these drugs is well defined,\(^9-14\) we are unaware of any report in the literature dealing with the effects of aforementioned drugs on the complement system.

In the present study, these drugs were tested for their intrinsic ability to inhibit complement activation _in vitro_ caused by the housedust mite allergen, _D. farinae_, in normal subjects as well as asthma patients who were skin-test-positive (STP) to this allergen.

**MATERIALS AND METHODS**

**Drugs**

Disodium cromoglycate cartridges were obtained from Unique Chemicals; hydrocortisone sodium succinate, from Pharmaceutical and Chemical Industries, India; diethylcarbamazine citrate, from Unichem Laboratories Ltd.; salbutamol, from Cipla, India; and aminophylline, from East India Pharmaceuticals Ltd.

**Allergen**

Housedust mite, *Dermatophagoides farinae* (1,000 protein nitrogen units (PNU/ml) was procured from Miles Laboratories Ltd., U.K.

**Patients**

Twelve patients with bronchial asthma who were strongly skin-test-positive to *D. farinae* took part in this study. The patients, who ranged in age from 15 to 44 years, were in a clinically stable phase. All drug used was suspended 24 hours before the study; however, all the drugs previously named were not tested in each patient.

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Controls

Five healthy subjects with no family history of asthma or any other allergic disorder formed the control group for this study. The age of controls ranged from 22 to 42 years.

Complement (C3) activation

Drugs at increasing concentrations were added to 0.5 ml of serum incubated at 37°C for 60 minutes. To this allergen, *D. farinnae* was added at a concentration of 100 μl/0.5 ml of serum; it was noted earlier that this concentration would give appreciable C3 activation. The entire amount was incubated at 37°C for one hour. This was then subjected to two-dimensional cross-electrophoresis using 2% rabbit anti-human C3 in 1% agarose (w/v) in order to assay the complement activation.

Two control samples were tested simultaneously, (a) the untreated serum and (b) the serum treated with allergen only. The untreated serum was pre-incubated in the absence of drugs at 37°C for two hours. The second control was pre-incubated at 37°C for one hour. To this allergen, *D. farinnae* was added (100 μl/0.5 ml serum) and was again incubated for one hour at 37°C before being subjected to two-dimensional cross-electrophoresis.

RESULTS AND DISCUSSION

Disodium cromoglycate and hydrocortisone

Disodium cromoglycate (2.5, 5, 10 and 20 μg) was tested separately with two samples of NHS and serum of six STP asthma patients. No activation of complement, i.e. conversion of C3 to C3b by *D. farinnae* was observed in the serum samples pretreated with 5, 10 and 20 μg of the drug in both the groups. But when a lower concentration (2.5 μg) of the drug was used, inhibition of complement activation was only partial (Fig. 1).

The effect of 12, 25, 35, 50, 250 and 500 μg of hydrocortisone was examined separately in two NHS and five STP patients. Pretreatment of serum with 50, 250 and 500 μg of drug resulted in a complete blockade of complement activation by *D. farinnae* in both the groups. But 12, 25 and 35 μg of the drug were not able to block the complement activation completely in normal subjects and asthma patients (Fig. 2).

Disodium cromoglycate and hydrocortisone were shown to block the complement (C3) activation induced by the common inhalant allergen, *D. farinnae*, as indicated by the absence of a C3b peak in the cross-electrophoretic patterns. This *in vitro* inhibition of the complement activity was only partial when low concentrations of these drugs were used. Both drugs behaved in an identical manner in normal subjects and STP asthma patients.

Inhibition of the release of injurious products (histamine, a slow reacting substance of anaphylaxis-
C3 INHIBITION BY DRUGS USE IN BRONCHIAL ASTHMA

Fig. 2 Cross-electrophoretic pattern of C3 from normal human serum (A) and the serum of a skin-test-positive (STP) asthmatic (B). a, Untreated serum; b, serum + D. farinae (100 µl); c, serum + hydrocortisone (12 µg) + D. farinae (100 µl); d, serum + hydrocortisone (500 µg) + D. farinae (100 µl).

SRS-A) has already been demonstrated in sensitised mast cells in vitro and in vivo by the use of disodium cromoglycate.9,10,16 Because of their beneficial and often life-saving effects, hydrocortisone and its analogues are used in the treatment of severe attacks of bronchial asthma.11 These compounds are extensively used to depress immunological responses; they possess a powerful, general, anti-inflammatory action. Although the mode of action of some of these drugs is well known, the effects of both disodium cromoglycate and hydrocortisone on the complement system have not been reported so far.

It is evident from the present study that disodium cromoglycate and hydrocortisone inhibit complement (C3) activation in vitro. Thus, they will also block the formation of anaphylatoxin (C3a and C5a) which in turn may spare the patient from the agony of asthmatic symptoms resulting from bronchospasm. We thus propose here another important mode of action of disodium cromoglycate and hydrocortisone via inhibiting the activation of the complement system in bronchial asthma patients.

Salbutamol, aminophylline and diethylcarbamazine citrate

Salbutamol at concentrations of 5, 25, 50 and 100 µg was not able to inhibit complement activation induced by the allergen in two normal subjects and four asthma patients. Similarly, amounts of 12.5, 25 and 50 µg of aminophylline had no inhibitory effect on complement activation in both the groups (one normal and three STP asthma patients). No inhibition of a conversion of C3 to C3b was observed in serum samples of one normal and two STP patients treated with 125, 250 and 500 µg of diethylcarbamazine citrate.

Bronchodilator treatment with selective β2 adrenergic receptor stimulants has been the conventional method of treatment of asthma in both adults and children. These drugs are known to stimulate adenyl cyclase thereby raising levels of cAMP which is turn stabilises the cell membrane and prevents histamine release. In the present study, salbutamol was not found to block C3 activation. Similar results were obtained when aminophylline, a smooth muscle relaxant of the xanthine type, was used. The theophylline group of drugs is known to act by an inhibitory effect on phosphodiesterase thereby increasing the levels of cAMP. From our study it appears that both salbutamol and aminophylline do not act through the complement system.

Diethylcarbamazine citrate, a commonly prescribed anthelminthic drug for the treatment of filariasis, has also been used with varying results in the treatment of bronchial asthma.14,17 The results observed in this communication clearly indicate that diethylcarbamazine does not inhibit the activation of the complement system induced by D. farinae. However, Staniunas and Hammerberg18...
recently reported that diethylcarbamazine enhanced the activation of the complement system caused by intact microfilariae of *Dirofilaria immitis* and their *in vitro* products.

ACKNOWLEDGEMENTS

We acknowledge the financial grant and other facilities received in support of this work from the Indian Council of Medical Research and the All-India Institute of Medical Sciences, New Delhi.

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