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INFLUENCE OF A BACTERIAL EXTRACT, BRONCHO-VAXOM, ON CLINICAL AND IMMUNOLOGICAL PARAMETERS IN PATIENTS WITH INTRINSIC ASTHMA

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Summary: *The effect of Broncho-Vaxom (BV), an orally administered lyophilized bacterial extract, has been investigated by a single-blind study on 50 patients with intrinsic asthma. In addition to conventional therapy, 25 patients received BV and the other 25 did not (in order to serve as controls). Both groups were matched with respect to age, sex, case history and pulmonary function. At the end of the 6-months trial, the clinical parameters showed a statistically significant improvement in the BV group ($p < 0.05$) when compared to the control group and this was accompanied by a significant reduction ($p < 0.05$) in the number and duration of asthmatic attacks. Moreover, the pulmonary function tests ($FEV_1/FVC\%$) and the bronchial responsiveness to methacholine challenge (PD_{20}) were significantly improved in the BV-treated patients ($p < 0.05$), while in the control group the values remained unaltered. Treatment with BV resulted in a significant increase in the serum levels of IgA, IgG and IgM ($p < 0.05$), with a statistically significant drop in the serum levels of IgE ($p < 0.05$). There was no change in the immunoglobulin levels in the control group. In the broncho-alveolar lavage fluid there was a decrease in the eosinophil count ($p < 0.05$), a correction towards normal value of the Th/Ts ratio ($p < 0.01$) and an increase in the secretory IgA/albumin in the BV group, while no changes were noted in the control group. These results denote that Broncho-Vaxom has a valuable effect both clinically and immunologically in patients with intrinsic asthma.*

Introduction

Intrinsic asthma, similar to all forms of asthma, is a chronic inflammatory disease which is characterized by the presence of airway hyper-responsiveness and airflow obstruction. In the adult population, it is associated with concomitant chronic bronchitis leading to post-infectious airway irritation that may trigger asthmatic attacks. The broncho-alveolar lavage (BAL) fluid from asthmatic patients has been found to contain an increased number of inflammatory cells, in particular eosinophils (1).

Current evidence suggests that attention should be directed towards the prevention of the precipitating factor (recurrent respiratory infections) in the management of this disease so as to prevent acute episodes of asthma (2). In this context, promising immunobiotherapeutic drugs containing purified bacterial extracts have been developed to regulate or enhance the body's natural immune mechanisms against infections.

Broncho-Vaxom® (BV; a product of OM Lab-

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oratories Ltd., Geneva, Switzerland) is an orally administered lyophilized bacterial extract containing immunogenic fractions derived from eight bacterial species that are commonly responsible for respiratory infections. Its therapeutic and preventive effects have been demonstrated in a number of studies in patients with recurrent respiratory tract infections with and without bronchial asthma (3–8).

The aim of the present trial was to assess in patients with intrinsic asthma the effect of Broncho-Vaxom on clinical manifestations of the disease, and the related immunological parameters.

Patients and methods

Fifty patients with intrinsic asthma who fulfilled the criteria of Scadding for bronchial asthma (9), and who were free from other diseases, were recruited to the study. The trial was conducted as a single-blind study, i.e. the investigators were unaware who was receiving the test product. A total of 25 patients, 18 males and 7 females (age range from 38 to 47 years) received, in addition to conventional therapy (antibiotics and bronchodilators), 1 capsule of Broncho-Vaxom for adults given daily before breakfast during the first month, followed by a 1-month rest, and then 1 capsule on each of the first 10 days of the 3rd, 4th and 5th months (BV group). The remaining 25 patients, 19 males and 6 females (age range from 40 to 48 years) received only conventional therapy (control group). All patients were observed monthly over a period of 6 months. Four patients from the BV group and 8 from the control group dropped out of the study (did not come to the last visit). Clinical and immunological examinations were performed at entry to the study and then 6 months later. The patients were asked to submit to the following examinations.

Clinical. Case history (cough, expectoration, dyspnoea and wheeze) was recorded, followed by a chest examination. The symptoms and signs were rated as improvement, stabilization or worsening.

Lung function. All patients underwent lung-function tests and measurement of the provocative dose (PD_{20}) of inhaled methacholine that causes a 20% reduction in the FEV_1, using a MEFAR MBB dosimeter. The expirogram, and particularly the forced expiratory volume in the first second (FEV_1), is the most widely used test in asthmatics (10) as it is relatively sensitive and has a low coefficient of variation (11). Moreover, the percentage increase in the FEV_1 after inhaling a bronchodilator (2 puffs of salbutamol) was also tested at the start of the trial.

Immunological. The following examinations were carried out.

a) The concentration of serum immunoglobulin A, G and M were determined by single radial immunodiffusion (12).
b) The concentration of total serum IgE was determined by an enzyme immuno-assay (13).
c) Leukocyte migration inhibition test (14) using candidin (1:10000) as antigen. A migration index (MI) is established by dividing the mean migration of leukocytes in the presence of antigen by the mean migration of leukocytes in the absence of antigen.
d) T-cell subsets: T-helper (Th) and T-suppressor-cytotoxic (Ts), using monoclonal antibodies of the OKT series (OKT_4 for Th and OKT_8 for Ts) by an indirect immunofluorescence technique (15).
e) Broncho-alveolar lavage (BAL) including:
   - secretory IgA and albumin measured by single radial immunodiffusion (12);
   - T-cell subsets, Th and Ts, determined by an indirect immunofluorescence technique (15);
   - total and differential cell count; cell viability was determined by Trypan blue.

Statistical analysis. The statistical analysis included the chi-squared test, Student t-test and pair-differences test.

Results

Effect on clinical parameters

At the end of the 6-months period, 76% of the patients in the BV group showed an overall improvement in clinical parameters against 47% in the control group (Table I).

The difference between the two groups was statistically significant ($p < 0.05$).

There was also a significant decrease in the number and duration of asthmatic attacks ($p < 0.05$) in the BV group at the end of the study (Table II), while there was only a significant decrease in the duration of asthmatic attacks in the control group ($p < 0.05$).

Table I *Clinical evaluation of patients' improvement in the BV and control groups at the end of the study.*

Parameters	BV group ($n = 21$)		Control group ($n = 17$)	
	No. of patients	Percentage	No. of patients	Percentage
Cough	16	76	8	47
Sputum colour	15	71	9	53
Sputum amount	15	71	9	53
Dyspnoea	15	71	7	41
Wheeze	17	81	7	41
Mean of overall clinical improvement	16	76*	8	47

* $p < 0.05$ compared to control group.

Effect on lung function

At the beginning of the study, the pulmonary function was comparable in both groups ($p > 0.05$), as determined by the forced expiratory volume in one second (FEV_1), the FEV_1/FVC (forced vital capacity) and the percentage improvement of FEV_1 after bronchodilatation with salbutamol. At the end of the study, the FEV_1 in the BV group passed from 1.75 ± 0.71 litre to 2.24 ± 0.86 litre, i.e. an increase of 28%, which is statistically significant ($p < 0.05$). In the control group, the increase in the FEV_1 was only 14% (Table III). The difference between the two groups was also significant ($p < 0.05$). The PD_{20} (provocative dose of inhaled methacholine that produces a 20% reduction in the FEV_1) was 111.3 ± 95.7 µg in the BV group and 149.8 ± 163.5 µg in the control group at the start of the trial (n.s.). At the end of the 6-months period, the PD_{20} in the BV group was more than double the initial value ($p < 0.05$), while remaining practically unchanged in the control group (n.s.) (Table III).

Effect on immunological parameters

Serum immunoglobulin concentrations were

Table II *Number and duration of asthmatic attacks before and at the end of the study.*

Parameters	BV group ($n = 21$)		Control group ($n = 17$)	
	Before	After 6 months	Before	After 6 months
No. of attacks per month	12.5 ± 11.1	4.9 ± 7.8*	15.3 ± 12.8	10.1 ± 9.0
Duration of attacks (h)	12.9 ± 13.0	5.5 ± 10.3*	5.8 ± 5.3	2.3 ± 3.1*

$p < 0.05$ compared to initial values.

Table III *Lung-function evaluation and bronchoprovocation challenge in the BV and control groups before and at the end of the study.*

Parameters	BV group ($n = 21$)	Control group ($n = 17$)
FEV_1/FVC (%) at entry	62.05 ± 9.78	66.3 ± 11.10
FEV_1 (litre) at entry	1.75 ± 0.71	1.58 ± 0.44
FEV_1 after bronchodilatation with salbutamol	2.09 ± 0.48	1.90 ± 0.51
% improvement	19.4	20.5
FEV_1 after 6 months	2.24 ± 0.86*	1.80 ± 0.56
% improvement in FEV_1	28	14
% of marked improvements (>30% FEV_1)	55	40
PD_{20} to methacholine before treatment (µg)	111.3 ± 95.7	149.8 ± 163.5
PD_{20} to methacholine after treatment (µg)	254.7 ± 357.4*	150.8 ± 137.1

FVC = forced vital capacity.
PD_{20} = provocative dose that produces 20% reduction in FEV_1.
* $p < 0.05$ compared to initial values.

comparable in both groups at the start ($p > 0.05$). After BV treatment, the levels of IgA, IgG and IgM showed a statistically significant increase ($p < 0.005$) associated with a significant decrease in the IgE levels ($p < 0.005$). There were no significant changes in the Ig levels in the control group ($p > 0.05$) (Table IV).

In both groups, the T-cell subsets in peripheral blood (Th/Ts ratio) and the leukocyte migration inhibition test determined as the migration index (MI) were comparable at the start of the trial (Table V). In BV-treated patients, the Th/Ts ratio and MI were statistically significantly reduced ($p < 0.05$ and $p < 0.005$ respectively). These values remained unchanged in the control group ($p > 0.05$).

BAL analysis peformed initially showed the absence of statistical differences between the two groups of the study ($p > 0.05$). After treatment there was no difference in all cell counts in both groups except for a significant decrease in the percentage of eosinophils ($p < 0.05$) in the BV group (Table VI). Moreover, in the BV-treated patients, the Th/Ts ratio increased significantly ($p < 0.01$) as well as the S-IgA/albumin ($p < 0.005$). No significant changes were observed in the control group after treatment (Table VI).

Discussion

Clinical follow-up of cases has shown that there was an overall improvement in both groups (Table I). However, the degree of improvement was more marked in BV patients (76%) and was statistically significant when compared to controls (47%). Also, analysis of each symptom separately revealed a substantial improvement in the BV group. Moreover, a significant reduction in the number and duration of asthmatic attacks was also noted in the BV-treated patients (Table II). Lung-function tests (Table III) have shown that all cases at the start

Table IV *Serum immunoglobulin levels before and after treatment in BV and control groups.*

Parameters	BV group ($n = 21$)		Control group ($n = 17$)	
	Before	After 6 months	Before	After 6 months
IgA (mg/dl)	160 ± 18	200 ± 22*	145 ± 19	150 ± 16
IgG (mg/dl)	1200 ± 180	1500 ± 200*	1310 ± 190	1350 ± 200
IgM (mg/dl)	110 ± 25	148 ± 28*	118 ± 24	121 ± 25
IgE (IU/ml)	180 ± 20	120 ± 24*	165 ± 17	170 ± 20

* $p < 0.005$

Table V *T-cell subsets in peripheral blood and leukocyte migration inhibition test (determined as MI) in BV and control groups before and after treatment.*

Parameters	BV group ($n = 21$)		Control group ($n = 17$)	
	Before	After 6 months	Before	After 6 months
Th/Ts ratio	2.42 ± 0.20	1.8 ± 0.19*	2.36 ± 0.18	2.34 ± 0.17
MI	0.9 ± 0.12	0.6 ± 0.14**	0.86 ± 0.16	0.83 ± 0.20

* $p < 0.05$ ** $p < 0.005$

Table VI *BAL analysis in BV and control groups before and at the end of the study.*

Parameters	BV group ($n = 21$)		Control group ($n = 17$)	
	Before	After 6 months	Before	After 6 months
Total cell count ($\times 10^6$)	28 ± 32	20 ± 30	27 ± 31	28 ± 30
Cell viability %	84 ± 28	88 ± 26	86 ± 30	84 ± 20
Lymphocytes %	14 ± 16	15 ± 15	16 ± 15	15 ± 14
Macrophages %	65 ± 30	71 ± 26	66 ± 26	66 ± 25
PMN %	21 ± 32	14 ± 34	18 ± 33	19 ± 32
Eosinophils (% of PMN)	1.3 ± 0.6	1.0 ± 0.4*	1.2 ± 0.5	1.2 ± 0.4
Th/Ts ratio	1.10 ± 0.4	1.41 ± 0.4**	1.13 ± 0.4	1.15 ± 0.4
S-IgA/albumin	0.20 ± 0.2	0.46 ± 0.2***	0.18 ± 0.2	0.20 ± 0.2

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$.
PMN = Polymorphonuclear leukocytes.

of the trial had FEV_1/FVC less than 70%, thus supporting the diagnosis of asthma (10). The mean FEV_1 was comparable in both groups, indicating that BV and control groups were homogeneous.

At entry to the study, both groups showed a similar reversibility of FEV_1 after administration of bronchodilator (2 puffs of salbutamol) ($p > 0.05$). A 15 to 20% improvement in FEV_1 is expected following the use of bronchodilators (16, 17) (Table III).

At the end of the study, there was a significant improvement in the mean FEV_1 in the BV group (28%) as compared to the control group (14%) ($p < 0.05$). Furthermore, 55% of the BV group showed marked improvements in FEV_1; i.e. an increase of more than 30% in FEV_1 compared to 40% in the control group (Table III). Methacholine challenge is a reliable test for bronchial reactivity to distinguish asthmatics from normal subjects and has a lower incidence of side-effects when compared to histamine challenge (18). In asthmatics, PD_{20}, the provocative dose of inhaled methacholine to produce a 20% drop in FEV_1, is usually below 600 µg (19).

At the onset, both groups had a mean PD_{20} to methacholine much below the 600 µg and were comparable ($p > 0.05$) as regards bronchial response. There was no change in the PD_{20} at the end of the study in the control group ($p > 0.05$), while in the BV group PD_{20} showed a significant

increase in the mean value ($p < 0.05$) which was more than double the initial mean value (Table III). Moreover, three cases in this group exceeded the limit of 600 µg methacholine inhalation. These data indicate a decrease in hyper-reactivity in BV-treated patients.

As regards immunoglobulin levels (Table IV) there was a significant increase in IgA, IgG and IgM in the BV group after treatment, while total IgE decreased ($p < 0.005$). These changes were not apparent in the control group ($p > 0.05$). Normalization of IgE levels is coherent with BV immunomodulatory effect.

In the peripheral blood, the Th/Ts ratio (Table V) after BV treatment also followed a downward trend towards the normal value ($p < 0.005$) which ranges between 1.5 to 1.8 (8). This was not the case for the controls ($p > 0.05$). The mean value of MI (Table V) in the BV group also decreased significantly ($p < 0.005$), denoting an improved lymphocyte function after BV treatment. Again, there was no significant change in the control group ($p > 0.05$).

In the BAL fluid, the Th/Ts ratio in the BV-treated group, on the contrary, increased significantly ($p < 0.005$) (Table VI) to reach near normal values. There was also a decrease in BAL eosinophils % ($p < 0.05$), while no significant ($p > 0.05$) changes appeared in the control group.

BAL S-IgA/albumin (Table VI) increased after treatment with BV ($p < 0.005$), while the controls showed no variation ($p > 0.05$), demonstrating that by increasing secretory IgA synthesis BV contributes towards the improvement of an important factor in local lung defence.

Conclusions

It appears from this study that BV by means of its immunomodulatory effect improves both the local and systemic immune defence mechanisms, accompanied by a marked clinical improvement, thus confirming the drug's great therapeutic value in the management of bronchial infections and inflammation, that constitute a major risk factor for intrinsic asthma patients.

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