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Study on clinical effect and immunologic mechanism of infants capillary bronchitis secondary bronchial asthma treated with bacterial lysates Broncho-Vaxom

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Abstract. – OBJECTIVE: To study the clinical effects and immunologic mechanism of infant capillary bronchitis secondary bronchial asthma treated with bacterial lysates (Broncho-Vaxom OM-85BV).

PATIENTS AND METHODS: Between February 2013 and February 2014, 136 infant capillary bronchitis secondary bronchial asthma cases were chosen. This research was approved by Ethics Committee in our hospital and obtained the informed consent right from patients and guardians. Patients were divided into the control group (n = 62) and the observation group (n = 74) using random number table method. Patients in the control group were treated with normal glucocorticoid atomizing inhalation, aminophylline and antibiotic treatment. In the observation group besides the abovementioned treatment, we added Broncho-Vaxom OM-85BV, qd po for 10 days continuously and quitted it for 20 days. This continued for a total of 3 months. Follow-ups were set for about one year to compare the effects.

RESULTS: The onset frequency and duration of capillary bronchitis and asthma in observation group declined remarkably compared with control group and the differences were statistically significant ($p < 0.05$). The level of IL-17 and IL-4 in the observation group decreased significantly, whereas, the level of IL-10 and IFN-γ increased considerably. Differences were all statistically significant ($p < 0.05$). Peripheral blood CD4+ T lymphocytes in the observation group patients expressed lower levels of nicotinic acetylcholine receptors α7 (α7nAChR) compared to the control group. Then difference was statistically significant ($p < 0.05$).

CONCLUSIONS: Broncho-Vaxom OM-85BV reduced the onset of infant capillary bronchitis secondary bronchial asthma, relating to the reduced inflammation reaction. It also regulated the immunologic function of Th1/Th2, and lowered the α7nAChR level.

Key Words:
Bacterial lysates, OM-85BV, Infant capillary bronchitis, Bronchial asthma, acetylcholine receptor alpha 7.

Introduction

Capillary bronchitis and bronchial asthma are two important asthmatic diseases in the infantile period, and they are closely linked[1]. Recurrent respiratory virus infection and immune dysfunction are the main cause of infant capillary bronchitis. Prior studies[2] suggested that the prevalence of repeated wheeze in infants with capillary bronchitis is 68% and the prevalence of asthma is 30%. Wheezing (temporary wheeze) in infants below one-year-old, may continue until they are three. Current treatment for this condition is limited to prevention of asthma caused by viral infection. OM-85 BV (Broncho-Vaxom; OM Pharma, Geneva, Switzerland) preparation contains lysates of eight bacterial pathogens (in equal parts) of the most often encountered microorganisms in respiratory tract infections. Broncho-Vaxom was shown to affect innate immunity influencing CD4 + T lymphocytes macrophages and neutrophils activity and proinflammatory cytokines production, as well as acquired immune responses regulated by lymphocytes and synthesis of immunoglobulins. Bacterial lysates reduce inflammation reaction and the occurrence of infant capillary bronchitis and bronchial asthma[3]. The evidence-based medicine indicates that OM-85BV is a safe and effective method of therapy[4]. The study tried to discuss further the possible mechanism involved in this method of therapy.

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Patients and Methods

Patients

Between February 2013 and February 2014, 136 infant capillary bronchitis, secondary bronchial asthma cases were chosen. The diagnostic criteria used to determine capillary bronchitis and bronchial asthma were the journals of pediatrics and related guidelines. Inclusion criteria (1) all patients conformed to diagnostic criteria; (2) Patients and the guardians signed informed consent forms and showed good compliance; (3) All patients had perfect follow-up data. Exclusion criteria: (1) Patients whose capillary bronchitis was not caused by respiratory viral infections and capillary bronchitis secondary bronchial asthma; (2) Infants congenital malformations, hypoevolutism and dementia cases; (3) Cases with other diseases associated with respiratory system, viscera dysfunction of systems such as heart, liver and kidney were all excluded.

The study was approved by Ethics Committee in our Hospital and we obtained informed consent right from patients and guardians. Patients were divided into control group (n = 62) and observation group (n = 74) using random number table method.

In our control group, there were 33 males and 29 females, aging from 7 months to 5 years with an average age of (2.2 ± 0.4) years; the course of the disease was 2 days to 3 months (average = 26.8 ± 5.5 days). All cases were exclusively breast fed; we had 50 cases of full-term deliveries, 12 cases of cesareans. In the observation group, there were 38 males and 36 females, aging from 8 months to 4.5 years (average = 2.3 ± 0.6 years); the course of disease was 3 days to 4 months (average = 28.7 ± 6.3 days). All patients were exclusively breast fed; we had 61 cases of full-term deliveries and 13 cases of cesareans. When comparing the general information of infants in the two groups, the difference showed no statistical difference ($p > 0.05$).

Therapeutic Method

The control group was treated with normal glucocorticoid atomizing inhalation, aminophylline and antibiotic. In the observation group we applied the same treatment as the control group and to that we added Broncho-Vaxom OM-85BV, qd po for 10 days continuously and after 10 days we stopped it for 20 days. In total we continued this treatment for 3 months. We set follow-up visits for about one year later in order to compare the effects.

Observation Indexes and Test Methods

We compared and analyzed the differences in the levels of IL-17, IL-4, IL-10 and IFN-γ in serum and the post-treatment occurrences as well as the duration of capillary bronchitis and bronchial asthma. We also verified the differences in the expression levels of nicotinic acetylcholine receptor alpha 7 (alpha 7 nachr) in CD4+ T cells isolated from peripheral blood.

The levels of IL-17, IL-4, IL-10 and IFN-γ in serum were tested using Enzyme-linked immune detector (Model550 type, Bio-Rad, Hercules, CA, USA). The details of the protocol used were as follows, venous blood (6 ml) was taken and 4 ml EDTA was added to it as an anticoagulant. Samples were centrifuged and kept at -20°C. The separation of mononuclear cells was done from the ultra clean bench. To start the experiment, the sample were thawed and placed on microporous plate frame followed by injection of 90 mu L diluted buffer and l0 μL sample into the reaction hole of package resistant to IL-17, IL-4, IL-10 hole and IFN-gamma antibody. We l00 μL standard into the corresponding reaction hole, sealed the plate and incubated for 60 min at room temperature. The plate was rinsed three times with 300 μL washing buffer and then pat dried it using bibulous paper. An anti IgE biotin (100 μL) was added to all holes, and the plates incubated for 60 min at room temperature after being sealed. The plates were then washed and 100 μL horseradish peroxidase (HRP) and a chain enzyme avidin combination were added. The plates were incubated in dark for 30 min at room temperature. Subsequently, the plates were washed and 100 μL of tetramethylbenzidine (TMB) substrate was added in the holes followed by 30 min incubation at room temperature. According to the protocol, we added 100 μL reaction terminating liquid. The density was measured at 450 nm wavelength.

α7nAChR testing instrument used was a flow cytometry instrument (Australia Coulter Company's product (Gladesville, Australia), type EPICS XL, standard System IITM software). The reagent was $CD4^+$ T lymphocyte separation of magnetic beads Miltenyi Biotec, α7nAChR the first resistance, α7nAChR the second resistance (FITC) (Abcam Co., Cambridge, MA, USA). The specific process was as follows: 5 ml EDTA anticoagulant was diluted with 1:1 PBS (phosphate buffered saline). 5 ml lymphocyte stratified fluid (Ficoll) taken from centrifuge tube and 10 ml diluted blood was added to stratified fluid

gently. It was centrifuged at 300 g for 20 min and the fluid was separated into many layers. At the bottom, we had red blood cells; in the middle, we had stratified fluid and at the top part, we had plasma. There was a thin and dense white layer between the plasma layer and the stratified fluid, which was the single nuclear cell layer. We inserted a capillary suction into the single nuclear cell layer and collected the layer and transferred it into another sterile tube. Single nuclear cells were counted. We added 80 µl PBS, BSA and EDTA and re-suspended the cells. We added 40 µl of CD4 cell magnetic beads into every 10^7 single nuclear cell, blending them at 4 to 8°C for 15 min. Then, we added l ml/10^7 cell suspension and washed the cells at 4 to 8°C, followed by centrifugation for 15 min at a speed of 2500 r/min. 500 µl of cell suspension was added into the re-suspended cells. Separation column was prepared and cell suspension was added into the column, using 500 µl buffer to wash the column three times. The column was moved from the magnetic field into another tube, using 1 ml buffer to wash it thoroughly. CD4+ T lymphocytes were collected and counted and their activity was tested. PBS was used to wash the dye (5 min at a time) and CD4+ labeled cells were collected.

PMA (20 ng/ml), ionomycin (l µg/ml) and BFA/monensin (2 nmol/ml) was added and incubated for 4 h. We then added α7nAChR primary antibody (0.5 ul/test) and incubated the plates in dark at 4°C for 20 min followed by centrifugation at 190 g for 5 min, rinsing (twice) and cells were re-suspension. α7nAChR secondary antibody (1.0 µl/test) was added followed by incubation in dark at 4°C for 30 min. Samples were fixed with 4% paraformaldehyde for 15 min and 0.1% saponin in the membrane for 10 min. APC Anti-human IL-17AAb was added (l0 µl) followed by 1 h incubation at 4°C in dark at room temperature. After rinsing the samples, they were analyzed with BD flow cytometry. Hydrogen ions were used for excitation and wavelength was 488 nm, scattering bandpass filter FITC was (530 ± 10) nm and PE was (575 ± 10) nm. We had framing lymphocyte area in forward angle and lateral angular scattering two-dimensional Dot Plot diagram. Boundary was defining in accordance to a non-specific light intensity of isotype and determining double negative, double positive and single positive areas to obtain 20,000 lymphocytes and the percentage of each area was calculated.

Statistical Analysis

We used SPSS 19.0 statistical software (SPSS Inc., Chicago, IL, USA) for data analysis. Measurement data were reported as mean ± standard, a *t*-test was adopted for comparison among groups, enumeration data were represented by cases or percentages. χ^2-test was used for comparison among groups. $p < 0.05$ was considered statistically significant.

Results

Comparison of the Onset Frequency and Duration of Capillary Bronchitis and Asthma

No significant differences were found when onset frequency and duration of the disease were compared within one year before the treatment patients with capillary bronchitis and asthma in the two groups ($p > 0.05$). After treatment, the onset frequency and duration of the disease in both groups all declined compared to the before treatment results. However, the observation group showed a more significant decline compared with the control group and the difference was statistically significant (Table I).

Table I. Comparison of the onset frequency and duration of capillary bronchitis and asthma.

Group	Pre-treatment				Post-treatment			
	Occurrence of capillary bronchitis	Duration time (d)	Occurrence of asthma	Duration time (d)	Occurrence of capillary bronchitis	Duration time (d)	Occurrence of asthma	Duration time (d)
Control group	23.5 ± 4.2	6.8 ± 1.2	14.4 ± 3.2	5.9 ± 1.4	17.9 ± 2.9	4.7 ± 0.8	10.2 ± 1.4	4.5 ± 0.7
Observation group	25.8 ± 4.5	7.0 ± 1.3	15.6 ± 3.3	6.1 ± 1.5	6.8 ± 1.6	3.3 ± 0.6	4.5 ± 0.9	2.9 ± 0.3
t	0.526	0.128	0.329	0.747	5.526	3.624	5.926	3.757
p	0.182	0.327	0.526	0.419	0.037	0.042	0.032	0.039

Table II. Comparison of the level of IL-17, IL-4, IL-10 and IFN-γ (ng/L) in the serum.

Group	Pre-treatment				Post-treatment			
	IL-17	IL-4	IL-10	IFN-γ	IL-17	IL-4	IL-10	IFN-γ
Control group	6.6 ± 1.2	77.5 ± 150.6	236.4 ± 56.4	869.7 ± 94.7	6.4 ± 1.3	73.2 ± 16.2	240.9 ± 60.2	897.8 ± 102.4
Observation group	6.9 ± 1.4	79.6 ± 170.4	214.5 ± 48.3	854.2 ± 96.5	3.7 ± 0.9	48.8 ± 10.5	336.4 ± 63.4	1246.5 ± 121.3
t	0.527	0.624	0.329	0.718	6.402	5.827	5.425	5.589
p	0.341	0.506	0.637	0.629	0.027	0.034	0.039	0.037

Comparison of the Level of IL-17, IL-4, IL-10 and IFN-γ in the Serum

The levels of IL-17, IL-4, IL-10 and IFN-γ in the two groups before treatment demonstrated no significant differences in both groups ($p > 0.05$). After treatment, the improvement of the control group was not noticeable, whereas, compared with the before treatment, the levels of IL-17 and IL-4 in the observation group was lower and the levels of IL-10 and IFN-γ were higher. Differences between groups were statistically significant ($p < 0.05$) (Table II).

ofα7nAChR Expression Level

Before treatment, the difference in the expression level of α7nAChR in two groups did not show any statistical significance ($p > 0.05$). After treatment, the change in the control group was not noticeable, while α7nAChR expression level in the observation group was lower significantly. Differences between the two groups were statistically significant ($p < 0.05$) (Table III).

Discussion

According to previous studies[5], immune dysfunction was closely related to capillary bronchitis and bronchial asthma and other infant asthmatic diseases. Thl/Th2 was unbalanced, expression and release of Th2 cytokines were increased while expression and release of Th1 cytokines were comparatively inhabited. The infantile period is a special physiological stage for the human body, in which the development of the respiratory system is incomplete and the immune system is immature. However, repeated respiratory infections can lead to a significantly downward trend of cellular and humoral immunity[6].

Clinical manifestations of the first onset of capillary bronchitis include typical bronchitis asthma, chronic inflammation of the airway which become more serious by time and gradually forms into airway hyper-responsiveness. Isolated infants suffer from airway plasticization and deterioration of lung function. Thus, a capillary bronchitis may be the early signal of bronchial asthma[7]. More and more people consider infants with capillary bronchitis who suffer from, as high-risk population for bronchial asthma.

Glucocorticoid inhalation or Montelukast can improve symptoms and lower the deterioration of infant's asthma, but the continuous use cannot reduce the total onsets of asthma[8]. It was revealed that the continuous use of the preventive anti-inflammatory drugs had no positive effects on such onsets[7]. Broncho-Vaxom OM-85BV has eight types of common bacteria (*Haemophilus influenzae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Neisseria catarrhalis*, grass green sphaerita, pyogenic stretococcus, *Klebsiella pneumoniae* and smelly nasal white clay). The specification of children capsule has 3.5 mg/grains, being taken orally. OM-85BV enters the human blood, and expedite the T lymphocyte cycle and enhance its activity. Mainly Th1 cell activity is the most obvious result of this intervention[9]. In many European countries, OM-

Table III. Comparison of α7nAChR expression level (%).

Group	Pre-treatment	Post-treatment	*t*	*p*
Control group	86.7 ± 16.5	85.3 ± 18.4	0.998	0.127
Observation group	89.4 ± 17.3	54.2 ± 13.2	6.423	0.023
t	0.312	6.529		
p	0.639	0.021		

85BV has already been widely used for children and adults for more than 20 years. Some large-scale randomized clinical trials showed that OM-85BV reduced onsets of acute bronchial asthma by 25 to 50 percent. Huber et al[10] analyzed OM-85BV's immunoregulatory effects *in vitro* and *in vivo*, and showed that in both cases, OM-85BV increased the Thl IFN-Y related cytokines. Also, they showed that level of Th2 related cytokines decreased in those cells. The immunological protective function of OM-85BV may be linked to rising Th1 response ability. As a kind of immune enhancer, OM-85BV can strengthen immune function through enhancing humoral immunity by increasing the level of protective immune globulin and improving the function of macrophages to improve cellular immunity[11].

Our results showed that the onset frequency and duration of capillary bronchitis and asthma in the observation group decreased remarkably compared with the control group and the difference was statistically significant. The levels of IL-17 and IL-4 in the observation group declined significantly, while the levels of IL-10 and IFN- γ increased significantly; α7 (α7nAChR) in the observation group was significantly lower than that in the control group and the difference was statistically significant. Some studies show that OM-85BV produced cumulative drug effects in the body and reduced the application of hormonal and short-acting 2-agonist. Rutishauser et al[12] revealed that the application of multivalent bacterial lysate was effective and safe. Moreover, the research pointed out that BV could protect preschoolers from wheezing caused by the virus. Such an effect worked mainly through effectively reducing the respiratory infection of recurrent wheezing preschoolers.

Conclusions

Broncho-Vaxom OM-85BV reduced the onsets of infant capillary bronchitis secondary bronchial asthma, relating to reduce inflammation reaction. It also regulated the immunologic function of Th1/Th2, and lowered the α7nAChR level.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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