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Analysis of the effect of bacterial lysate and the immunologic mechanism in treating infant bronchiolitis

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Abstract. – OBJECTIVE: We studied the effect of bacterial lysate and the immunologic mechanism in treating infant bronchiolitis.

PATIENTS AND METHODS: 124 infants were diagnosed with bronchiolitis were consecutively selected and randomly divided into control group and observation group, with 62 cases in each group. Conventional therapies were administered in the control group, while bacterial lysates were administered in the observation group. Therapeutic effects were compared after 14 days.

RESULTS: In the control group, the total effective rate experienced prominent increase and the healing period was shortened. The differences were statistically significant ($p < 0.05$). Comparison of the reverse reactions in two groups showed no statistical difference ($p > 0.05$). Levels of serum IFN-γ, IL-4, NF-κB and KBD-1 after treatment showed no prominent changes in the control group. Levels of IFN-γ and Hbd-1 increased while levels of IL-4 and NF-κB decreased in the observation group; the differences were statistically significant ($p < 0.05$). Comparisons of the indexes above mentioned after treatment in the two groups showed significant differences ($p < 0.05$). Levels of IgA, IgG and IgM after treatment in the control group showed no changes, as well as the level of IgM in the observation group. Levels of IgA and IgG after treatment in the observation group prominently increased and were higher than that in the observation group; the differences were statistically significant ($p < 0.05$).

CONCLUSIONS: Bacterial lysate can improve the therapeutic effect of infant bronchiolitis; it can also improve the level of certain cytokines, immunoglobulins, and strengthening immunity.

Key Words:
Bacterial lysate, Infant, Bronchiolitis, FN-Ð, IL-4, NF-ÐB, KBD-1, IgA, IgG, IgM.

Introduction

90% infants have been infected with bronchiolitis in their first two years. The pathogen is mainly respiratory syncytial virus (RSV) and 40% of infected infants developed lower respiratory infection[1]. The causes of contracting the infection include cesarean delivery, premature delivery, birth injury, low position of the fetus, ischemia-hypoxia, congenital low immunity, improper breast feeding or artificial feeding, congenital abnormal airway, cross infection and so on. Clinical therapies mainly include oxygen inhalation, aerosol inhalation of bronchodilators or glucocorticoid, appropriate amount of fluid infusion, and if necessary, administration of antibiotic, RSV monoclonal antibody and other therapies[2,3]. Bacterial lysate (broncho-vaxom) is a frozen dry lysate of 8 different kinds of bacteria, which can stimulate the activity of macrophages, increase the composition of cytokine and secretion of immunoglobulins, and enhance the immunity of the body through strengthening the non-specific and specific immunity of the mucosal immune system, thus reducing the prevalence of respiratory tract infections[4,5]. Multiple studies indicate that bacteria lysate has beneficial therapeutic effect and enables less dosage of glucocorticoid administration when treating infants that are suffering from bronchial asthma with or without bacterial infection. This study investigated the effect of bacterial lysate and the immunologic mechanism in treating infant bronchiolitis in order to provide guidance for clinical application[6].

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Patients and Methods

Patients

124 infants diagnosed with bronchiolitis were selected by following the diagnostic criteria of bronchiolitis published by American Academy of Pediatrics in 2006. Infants allergic to bacterial lysate, recently transfused or treated with immunomodulators, with uncontrolled acute fever, diagnosed with congenital aplasia, diagnosed with congenital low immunity, poorly cooperating infants, infants unable to take drugs, with severe dyspnoea and those that were predicted to survive less than 1 month were excluded. This study was approved by the Ethics Committee of our hospital and informed consent was obtained by the guardians of the infants. Infants were randomly divided into the control group and the observation group, with 62 cases in each group. The control group included 36 boys and 26 girls with ages ranging from 2 months to 2.5 years, averaged at (1.0±0.3) years. 45 cases were born by eutocia and 17 cases by cesarean. 20 cases were born premature and 42 cases were full term delivery. Birth weight of the infants ranged from 1.8 to 3.2 kg, averaged at (2.7±0.6) kg. 40 infants were breast fed and 22 infants were artificially fed. Oxygen saturation of the infants ranged from 90% to 96%, averaged at (94.2±3.6) %. The observation group included 34 boys and 28 girls with ages ranging from 2.5 months to 2.0 years, averaged at (1.2±0.5) years. 43 infants were born by eutocia and 19 cases by cesarean. 21 cases were premature and 41 cases were both at full term delivery. Birth weight ranged from 1.6 to 3.0 kg, averaged at (2.5±0.7) kg; 42 cases were breast fed and 20 cases were artificially fed. Oxygen saturation ranged from 91% to 97%, averaged at (94.6±3.8) %. The baseline information of both groups was comparable.

Methods

The infected infants of both groups were treated by the same medical team following the standard medical guide and recommendation. The risk factors, possible cause of the disease and the degree of disease severity were judged preliminarily. The treatment methods included oxygen inhalation, aerosol inhalation of bronchodilators or glucocorticoid, anti-virus with ribavirin, chest physiotherapy, administration of antibiotic, RSV monoclonal antibody (palivizumab) if necessary, breast feeding (disinfection), appropriate hand disinfection and so on. Conventional therapies were administered in the control group; placebo was also given for comparison. Bacterial lysate (produced by OM Pharma, Geneve, Switzerland, number of Imported Drug License: S20100063, 3.5 mg/ pill) was administered to the observation group. Random, double-blind, and placebo comparisons were conducted, the drugs were administered by a pharmacist that did not participate in the clinical observation. The bacterial lysate and placebo were randomly numbered by a computer with 6 boxes as a group. The selected cases were treated with corresponding drugs after being randomly numbered by a computer. The appearance, color and taste of the bacterial lysates and placebos had no differences, and the doctors and the patients were unaware of the group they belonged to. The dosage of the drug referred to the past studies. In our center, infants less than 1-year-old took 0.875 mg/d at a draught in the morning after pulverized and dissolved once every day. Infants older than 1-year-old took 1.75 mg/d once every day and terminated after 14 days. Adversee reactions were noted, and the drug was withdrawn if necessary.

Observation Targets

The rate of therapeutic effect, healing period, adverse reaction of the drug and levels of serum IFN-γ, IL-4, NF-Kb, hBD-1, IgA, IgG and IgM were compared. Absolute disappearance of clinical symptoms, stable life signs and no recurrence after drug withdrawal indicate that the patient was healed. Relief of clinical symptoms, stable life signs and possibility of drug withdrawal for observation indicate effectiveness. No relief or worsening of clinical symptoms indicate ineffectiveness. The serum was testing accordingly: 5 ml cubital venous blood was drawn fast, with heparin applied, and centrifuged 2500 r/min for 10 min, the serum was segregated, and preserved and sent for testing together in -20°C. The levels of IFN-γ, IL-4, NF-Kb and hBD-1 were tested by ELISA. The kit was bought from American ADL Company and serum IgA, IgG and IgM were tested by immuno-nephelometry. The kit was bought from Shanghai Fosun Long March Medical Science Co., Ltd. The instructions of the kits were strictly followed.

Statistical Analysis

Software SPSS 20.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis and measurement data was presented by mean ± standard deviation. Comparison between groups was analyzed by independent sample *t*-test. The com-

Table I. Comparison of the therapeutic effective rate, healing period and the reverse reaction of the drugs.

Group	Case	Healed	Effective	Ineffective	Total effective rate	Healing period (d)	Hypersensitiveness	Abdominal pain and diarrhea	Reverse reaction rate
Control group	62	20 (32.3)	329 (51.6)	10 (16.1)	52 (83.9)	8.3±1.4	0	1	1 (1.6)
Observation group	62	26 (41.9)	33 (53.2)	3 (4.8)	59 (95.2)	5.6±1.2	1	1	2 (3.2)
t (χ^2)					4.211	6.320			0.000
p					0.040	0.029			1.000

Table II. Comparison of the levels of serum IFN-γ, IL-4, NF-κB and hBD-1.

Group	IFN-γ (pg/ml)		IL-4 (pg/ml)		NF-κB (pg/ml)		hBD-1 (μg/ml)	
Control group	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control group	236.4±35.3	253.5±36.7	124.5±26.8	103.6±22.4	56.8±12.0	50.7±12.8	6.8±1.2	7.0±1.3
Observation group	232.8±32.6	365.7±35.9	130.6±30.5	82.5±21.3	60.2±13.6	42.3±13.2	6.6±1.3	8.2±1.5
t	0.231	7.524	0.265	7.132	0.345	7.625	0.532	7.459
p	0.659	0.016	0.724	0.019	0.823	0.013	0.496	0.015

parison within the group were made by paired t-test. Enumeration data was represented by cases or (%). Comparison between groups was analyzed by (regulated) χ^2-test. $p<0.05$ indicated significant statistical difference.

Results

Comparison of the Therapeutic Effective Rate, Healing Period and the Adverse Reaction of the Drugs

In the control group, total effective rate experienced prominent increase, and the healing period was shortened. The differences were statistically significant ($p<0.05$). Comparison of the adverse reactions in two groups showed no statistical significance ($p>0.05$) (Table I).

Comparison of the Levels of Serum IFN-γ, IL-4, NF-κB and hBD-1

Levels of serum IFN-γ, IL-4, NF-κB and hBD-1 before treatment in both groups showed no statistical significance ($p>0.05$). Indexes mentioned above after treatment showed no prominent changes in the control group and the levels of IFN-γ and hBD-1 increased while that of IL-4 and NF-κB decreased in the observation group. The differences were statistically significant ($p<0.05$). Comparisons of the indexes above mentioned after treatment in both groups showed significant differences ($p<0.05$) (Table II).

Comparison of the IgA, IgG and IgM

Levels of IgA, IgG and IgM in both groups before treatment showed no significant statistical differences ($p>0.05$). The indexes mentioned above after treatment in the control group showed no changes. The level of IgM in observation group was unchanged as well. Levels of IgA and IgG after treatment in the observation group prominently increased and were higher than that in the observation group; the differences were statistically significant ($p<0.05$) (Table III).

Discussion

The body is usually unable to develop long term or permanent immunity when infected with RSV, which leads to repeated or multiple infections. Repeated infections may also severely affect the normal growth and cognitive development of the infected infants[7]. At present, the application of bronchodilator remains a controversy. Researchers, including Gadomski et al[8], conducted a randomized controlled trail (RCT) of 8 items on the infected infants, which revealed

Table III. Comparison of the IgA, IgG and IgM (g/L).

	IgA		IgG		IgM	
Group	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control group	1.3±0.4	1.4±0.3	7.6±1.5	7.7±1.6	2.3±0.4	2.2±0.3
Observation group	1.3±0.5	1.6±0.5	7.5±1.6	9.0±1.8	2.3±0.5	2.2±0.3
t	0.521	6.235	0.463	6.649	0.823	0.238
p	0.639	0.037	0.728	0.034	0.139	0.634

that only 1/4 of the infants treated with bronchodilators made temporary improvement on clinical scores. The differences were not statistically significant. The treatment courses were neither affected with the bronchodilator. Glucocorticoid should not be used as the conventional therapeutic drug for bronchiolitis. 13 items of RCT conducted with 1198 infected infants were included in a systematic assessment of the effect of glucocorticoid on treating acute bronchiolitis in the database of Cochrane. The results[9] demonstrated that the length of stay, clinical score, respiratory frequency, saturation of blood oxygen, recurrence rate after first treatment and the hospitalization rate of the infected infants in the therapy group were not statistically different with that of the placebo group. Ribavirin should not be conventionally used with infants that were infected with bronchiolitis. A recent review[10] including 11 random clinical experiments analyzed the result of ribavirin treating lower respiratory infection (including bronchiolitis), and 9 researchers observed the influence of ribavirin on acute stage. Another 2 researchers evaluated that the symptoms of long-term gasp and the function of lung. As a result, 7 studies indicated improvement with ribavirin and another 4 indicated failure.

This study concluded that the total efficiency increased prominently and the healing period shortened in the observation group. The adverse reaction rate of both groups had no difference, which indicates the safety and effectiveness of the application of bacterial lysate. Levels of serum IFN-γ, IL-4, NF-κB and KBD-1 after treatment showed no prominent changes in control group, levels of IFN-γ and Hbd-1 increased while that of IL-4 and NF-κB decreased in the observation group. The differences were statistically significant. The results indicated that the bacterial lysate was able to strengthen the immune function of Th1 cell and weaken the immunity of Th2 cell. Disorder of Th1/Th2 balance has been known to play an important role in the occurrence of bronchiolitis and asthma in infants[11]. The increase of the cell factor secreted by Th1 cells can strengthen the cellular immune response and increase the therapeutic effect of drugs. NF-KB takes part in multiple cellular pathways, playing function on immune suppression, apoptosis and induction of immunity disorder[12]. hBD-1 is a kind of important innate immune molecule which not only plays a key part in innate immunity but also plays an even more important part in acquired immunity through the chemotaxis of the dendritic cell and T cell[13]. Levels of IgA, IgG and IgM after treatment in the control group showed no changes as well as the level of IgM in observation group. Levels of IgA and IgG after treatment in observation group prominently increased and were higher than that in the control group; the differences were statistically significant.

Conclusions

Bacterial lysate can improve the therapeutic effect of infant bronchiolitis, improve the levels of certain cytokines and immunoglobulins, and strengthen the immunity. However, it still lacks a large number of sample experiments for comprehensive evaluation of the application of bacterial lysate at present. At the same time, there is no unified judging standard of the applied dosage, channel and the application effect of the bacterial lysate in infants. The correct administration of bacterial lysate still needs further discussion and evaluation.

Conflict of interest

The authors declare no conflicts of interest.

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