IMMUNOMODULATING AND ANTI-RELAPSE EFFECTS OF OZONE THERAPY IN ATOPIC DERMATITIS IN PRESCHOOL AND PRIMARY SCHOOL CHILDREN

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The aim of the investigation was to study the state of immunologic responsiveness, immunomodulating and anti-relapse effects of ozone therapy in children with severe extended atopic dermatitis.

Materials and Methods. We examined 64 children (38 boys and 26 girls) aged 5–10 years with severe extended atopic dermatitis. Group 1 patients (n=33) received complex standard treatment, Group 2 (n=31) — complex therapy in combination with ozone therapy.

Results. Complex standard therapy resulted in complete, though short, clinical remission; and in remission the patients preserved the changed parameters of cellular and humoral components of immune system, nonspecific resistance and the levels of pro-inflammatory cytokines in blood serum; while the patients receiving complex therapy combined with ozone therapy were found to have more rapid improvement of clinical indices, normalization of the most parameters of immunologic responsiveness and a long clinical remission.

Key words: atopic dermatitis in children; ozone therapy; immunity; dermatitis remission.

Modern comprehensive conventional therapy in preschool and primary school children with severe extended atopic dermatitis does not always lead to a long-term clinical remission. Therefore new treatment methods for patients should be searched for.

In recent years ozone therapy with anti-inflammatory, analgesic, detoxifying, bactericidal, virucidal, antioxidant and immunomodulating effects has been successfully used in comprehensive treatment of a variety of diseases [1]. There is very little information in the literature concerning the effectiveness of ozone therapy for atopic dermatitis in adults [2–4], but there are no data on the results of its application in the specified disease in children.

The aim of the investigation was to examine the state of immunologic responsiveness, immunomodulating and anti-relapse effects of ozone therapy in children with a severe form of extended atopic dermatitis.

Materials and Methods. We examined 64 patients (38 boys and 26 girls) with extended severe atopic dermatitis aged 5–10 years ("infant form" of atopic dermatitis in accordance with the classification given in the Academic and research program "Atopic dermatitis in children: diagnosis, treatment and prevention", Moscow, 2000). Depending on the conducted treatment they were classified into two groups. Group 1 (n=33) underwent comprehensive conventional treatment. Parents of children with atopic dermatitis were recommended to create hypoallergenic conditions of life, to use health and beauty skin care with application of triple action emulsion "Emolium II" during daily bathing, and triple action moisturizing cream "Emolium II" after swimming, to apply skin cream "Elokom" to the affected areas (once per day within 7–10 days), to stay on an individual hypoallergenic diet with the exception of cause-significant and mandatory allergens, to take Claritin or Zirtek (within 2 weeks), vitamins A, E, B_5, B_6, B_{15}, to undergo treatment with Hylak forte, Linex and Creon. General patients of Group 2 (n=31) were administered the same treatment, but in combination with two courses of ozone therapy. The course of ozone therapy consisted of lubrication of the affected skin areas with the ozonated olive oil (two times a day within 15 days) and rectal insufflations of ozone-oxygen mixture (in a day, total of 8 sessions). Ozone generation was carried out by a synthesizer "А-с-ГОКСф-5-05-ОЗОН" (LEPSE "Electric Machine Plant", Open Joint Stock Company, Kirov, Russia). Olive oil was ozonated at a concentration of ozone in the ozone-oxygen mixture of 20 mg/l. Therapeutic dose at rectal insufflation was 75 mg per 1 kg of the patient weight. The volume of ozone-oxygen

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mixture per one rectal insufflation was calculated using the formula: *body weight* (*kg*)·75/20; average volume of ozone-oxygen mixture per a rectal insufflation constituted 70 ml, per one course — 560 ml. The first course of ozone therapy in children with atopic dermatitis was started from the first day of examination, the second course was conducted in 3 months. Patients receiving comprehensive treatment in combination with ozone therapy had no complications or adverse reactions.

To assess the state of immunity in patients with atopic dermatitis in the first 1–2 days of examination (exacerbation of the disease) and after 18–24 days from the start of examination (period of clinical remission) we determined the content of CD3, CD4, CD8, HLA-DR$^+$, CD16 and CD20 lymphocytes in the blood (method of indirect immunofluorescence using monoclonal antibodies LT3, LT4, LT8, MCA HLA-DR, LT16, LT20), the content of immunoglobulins G, A, M in blood serum (method of radial immunodiffusion using monospecific antisera), total content of IgE (enzyme-linked immunoelectrodiffusion essay — ELISA) and circulating immune complexes (CIC) in blood serum (uniform precipitation method with a solution of polyethylene glycol). In addition, we examined the phagocytic activity of neutrophils (PAN), phagocytic index (PI) and nitroblue tetrazolium test (NBT-test) in the neutrophil cytoplasm (latex particles were used as the phagocytic object), also we determined the content of interleukin-8 (IL-8) and tumor necrosis factor-alpha (TNF-α) in blood serum (ELISA method).

We compared the results of the study in the groups of patients with atopic dermatitis with the results of these studies in 83 healthy children of the same age living in the city of Kirov and the Kirov region (Russia). The processing of the received data was performed using the computer program Microsoft Office Excel Mac 2011, the results were expressed as M±m, where M — arithmetic mean, m — root mean-square deviation.

Results and Discussion. Among examined children with extended type of atopic dermatitis boys dominated (60%), moreover, all children had heredity for allergic diseases. The past history of the patients records signs of exudative-catarrhal constitutional anomalies, pyoderma, earlier infectious diseases (ARVI, bronchitis, pneumonia, etc.), and dyspeptic symptoms not connected with infection. All patients had signs of food and drug allergies, as well as signs of polyvalent sensibilization to domestic, epidermal and pollen allergens. Allergic skin inflammation in the majority of the children examined (91%) occurred in the first half, in the other children — in the second half of life. We observed an exacerbation of atopic dermatitis in patients every 1.5–2 months and more frequently and it was caused by the violation of the diet, exposure to cause-significant allergens or acute infectious diseases.

In the period of exacerbation of the disease both groups of patients with infant atopic dermatitis had marked changes in the parameters of immunologic responsiveness (Table 1, 2). Thus, Groups 1 and 2 of patients in the acute period of the disease had an increase in relative and absolute number of CD3 lymphocytes ($p<0.001$), in absolute number of CD4 lymphocytes ($p<0.05$; $p<0.01$) and in relative and absolute number of CD8 lymphocytes ($p<0.001$); a decrease in immunoregulatory index CD4/CD8 ($p<0.001$), in relative amount of HLA-DR$^+$ lymphocytes ($p<0.001$) and CD16 ($p<0.001$), an increase in the relative and absolute number of CD20 ($p<0.02$; $p<0.02$; $p<0.001$; $p<0.001$) in the blood.

However, Groups 1 and 2 of patients in exacerbation of the disease had increased levels of IgG ($p<0.001$) and IgM ($p<0.001$), a marked increase in the level of total IgE ($p<0.001$) in blood serum, a PAN rate increase ($p<0.01$; $p<0.02$), a decrease in the PI values ($p<0.02$) and NBT-test ($p<0.02$; $p<0.001$), a marked increase in IL-8 ($p<0.001$) and TNF-α ($p<0.001$) in blood serum. In this case, we did not detect a significant difference between the values of certain indicators of immunity in Groups 1 and 2 of children with atopic dermatitis in the period of exacerbation of the disease.

In the course of treatment both groups of patients showed improvement of health, normalization of appetite and sleep, reduction and disappearance of skin itching, of inflammatory changes in the skin and other clinical manifestations of the disease. In this case, Group 2 that received comprehensive

Table 1

Populations and subpopulations of lymphocytes in blood of patients with atopic dermatitis

Indices	Healthy children (n=83)	Patients, exacerbation period		Patients, remission period	
		Group 1 (n=33)	Group 2 (n =31)	Group 1 (n=33)	Group 2 (n=31)
CD3,%	64.10±1.25	75.52±2.48*	76.03±2.55*	73.45±2.48*	69.11±0.92
CD3,10^9/L	1.04±0.07	2.48±0.33*	2.39±0.42*	2.21±0.32*	1.63±0.18*
CD4,%	49.30±0.80	46.02±2.72	47.11±2.34	43.92±3.02	48.11±2.11
CD4,10^9/L	0.73±0.03	0.98±0.11*	1.02±0.10*	0.96±0.05*	0.76±0.09
CD8,%	25.50±0.50	36.11±1.72*	34.55±1.61*	30.23±1.22*	27.31±0.92
CD8,10^9/L	0.36±0.01	0.73±0.09*	0.82±0.11*	0.62±0.09*	0.41±0.08
Index CD4/CD8	2.10±0.06	1.27±0.17*	1.36±0.19*	1.45±0.23*	1.76±0.27
HLA-DR$^+$,%	19.50±1.06	12.55±1.37*	11.69±1.28*	13.26±1.48*	18.28±1.21
HLA-DR$^+$,10^9/L	0.33±0.02	0.40±0.07	0.37±0.08	0.41±0.09	0.34±0.05
CD16,%	18.20±1.95	10.92±2.00*	10.48±1.93*	16.92±1.78	15.82±1.48
CD16,10^9/L	0.37±0.05	0.40±0.03	0.38±0.05	0.43±0.06	0.37±0.08
CD20,%	9.30±0.77	14.11±0.65*	14.24±0.92*	13.64±1.03*	9.78±0.56
CD20,10^9/L	0.17±0.02	0.39±0.07*	0.37±0.08*	0.36±0.05*	0.24±0.07

* — statistically significant difference with indices in virtually healthy children; $p<0.05$–0.001.

Table 2

The levels of immunoglobulins and circulating immune complexes in serum, the indices of phagocytosis and the levels of cytokines in blood serum of patients with atopic dermatitis (M±m)

Indices	Healthy children (n=83)	Patients, exacerbation period		Patients, remission period	
		Group 1 (n=33)	Group 2 (n =31)	Group 1 (n=33)	Group 2 (n=31)
IgG, g/L	8.90±0.14	16.11±0.83*	15.24±0.96*	12.44±0.38*	10.03±0.55
IgA, g/L	0.86±0.03	1.02±0.21	1.15±0.22	1.11±0.26	0.98±0.07
IgM, g/L	1.10±0.04	1.92±0.22*	1.79±0.18*	1.62±0.09*	1.22±0.13
IgE, IU/ml	151.00±46.20	545.17±61.22*	578.92±60.32*	524.17±40.82*	290.11±37.82*
CIC, ODU	0.070±0.004	0.074±0.011	0.072±0.009	0.068±0.012	0.072±0.011
PAN, %	66.70±1.11	78.62±4.21*	79.23±3.92*	77.24±3.02*	65.24±1.37
PI	10.80±0.17	7.92±0.41*	8.03±0.32*	9.52±0.28*	9.92±0.45
NBT-test, %	17.70±0.69	10.03±0.64*	9.85±0.73*	15.62±1.02*	18.11±0.56
IL-8, pcg/ml	8.11±0.30	17.36±0.58*	16.21±0.72*	16.98±0.71*	6.28±0.71
TNF-α, pcg/ml	1.86±0.09	9.27±0.72*	8.86±0.73*	8.42±0.68*	3.64±0.52*

* — statistically significant difference with indices in virtually healthy children; p<0.05–0.001.

treatment in combination with ozone therapy was found to have a complete clinical remission in 18.4±1.2 days from the beginning of treatment, i.e. 3.7 days earlier than Group 1 that received conventional therapy in 22.1±0.9 days from the beginning of treatment.

Studies carried out following a complete clinical remission revealed ambiguous changes in indicators of immunologic responsiveness in patients with atopic dermatitis receiving different treatment. Thus, Group 1 of patients with atopic dermatitis who underwent conventional treatment in the period of clinical remission demonstrated shifts of immunity parameters, less marked, but similar in nature to those that were identified in the period of exacerbation. A different pattern of changes in the immune system was revealed in Group 2 that received comprehensive treatment in combination with ozone therapy (See Table 1 and 2). These patients demonstrated a change in three factors only: an increase in the absolute number of CD3 lymphocytes (p<0.001) in the blood, an increase in the total IgE (p<0.001) and TNF-α (p<0.001) in the blood serum, while other immunity parameters did not differ significantly from those of healthy children.

The follow-up study showed that children in Group 1 receiving comprehensive conventional treatment in 3.1±0.3 months from the start of clinical remission had reappeared signs of allergic inflammation of the skin in the form of eczema and papular rash, as well as skin itching. The children, who along with a comprehensive conventional treatment had undergone two courses of ozone therapy, did not have clinical signs of disease exacerbation for 10.4±0.4 months.

Conclusion. Children with severe form of extended atopic dermatitis during exacerbation period of the disease were found to have violations of cellular and humoral components of the immune system, reduced non-specific resistance and high levels of pro-inflammatory cytokines in the blood serum. Children who received comprehensive conventional treatment preserved the changes in immunologic responsiveness upon the occurrence of clinical remission. It means that the treatment was ineffective and shows predisposition of the organism to an allergic reaction and disease recurrence. Inclusion of ozone therapy in the comprehensive treatment of patients results in a more rapid onset of clinical remission and normalization of most parameters of immunologic responsiveness. Two courses of ozone therapy at an interval of three months provide clinical remission, the duration of which exceeds three times the remission after comprehensive conventional treatment.

Data received in the course of clinical observation and special studies indicate high immunomodulatory and anti-relapse effectiveness of comprehensive treatment in combination with ozone therapy in extended severe atopic dermatitis in preschool and primary school children.

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