OZONE THERAPY CAPABILITIES IN CORRECTION OF NONSPECIFIC ANTIMICROBIAL RESISTANCE IN INFANTILE ATOPIC DERMATITIS

UDC 616.5–001/.002:615.015.26:612.014.464
Received 28.01.2014

© **Ya.Yu. Illek,** D.Med.Sc., Professor, Head of the Department of Pediatrics[1];
A.V. Galanina, D.Med.Sc., Professor, the Department of Pediatrics[1];
N.V. Isaeva, PhD, Senior Research Worker, the Immunohematology Laboratory[2];
E.V. Suslova, PhD, Tutor, the Department of Pediatrics[1];
T.N. Rybakova, Postgraduate, the Department of Pediatrics[1];
E.A. Fedyaeva, Postgraduate, the Department of Pediatrics[1];
V.V. Kuznetsova, Postgraduate, the Department of Pediatrics[1];
G.A. Zaytseva, D.Med.Sc., Professor, the Head of the Immunohematology Laboratory[2]

[1]Kirov State Medical Academy, Karl Marx St., 88, Kirov, Russian Federation, 610000;
[2]Kirov Scientific Research Institute of Hematology and Transfusion, Federal Bio-Medical Agency, Krasnoarmeiskaya St., 72, Kirov, Russian Federation, 610027

The aim of the investigation was to assess ozone therapy capabilities in the correction of nonspecific antimicrobial resistance in infantile atopic dermatitis.

Materials and Methods. 65 children (40 boys and 25 girls) aged from 8 months to 3 years with medium-severe forms of general atopic dermatitis were under observation. Group 1 patients (n=33) received complex conventional therapy, group 2 patients (n=32) — complex treatment combined with ozone therapy. We studied the content of anti-α-staphylolysin in blood serum, phagocytosis indices, the functional state of toll-like receptors (TLR-2 and TLR-6).

Results. Complex conventional therapy resulted in complete, though short-term clinical remission. When clinical remission occurred, the changes in the parameters of nonspecific antimicrobial resistance in patients with atopic dermatitis persisted. The patients receiving complex treatment combined with ozone therapy were found to have more rapid positive dynamics of clinical indices, normalization of anti-α-staphylolysin content in blood serum, phagocytosis indices, and marked function activation of toll-like receptors 2 and toll-like receptors 6, and the occurrence of a long-term clinical remission.

Key words: atopic dermatitis; nonspecific antimicrobial resistance; ozone therapy.

The most common complication of atopic dermatitis in children is the secondary infection of the skin. Most children with atopic dermatitis are also known [1–4] to have their skin colonized with staphylococcus, which may cause exacerbation of the disease and maintain a chronic course of allergic skin inflammation by secretion of superantibodies stimulating nonspecific activation of T-lymphocytes and macrophages, proinflammatory cytokine synthesis.

High efficiency of ozone therapy in many acute and chronic diseases [5], atopic dermatitis in adults [6, 7], as well as atopic dermatitis in infants, children and adolescents [8–11], the absence of contraindications to application, the absence of adverse reactions and complications with the proper dosing of administered ozone [5] are known to be reported. It was of interest to study the clinical indices, parameters of nonspecific antimicrobial resistance and functional activity of toll-like receptors in children with atopic dermatitis, who underwent a complex treatment which included ozone therapy. Similar studies have not been conducted so far.

The aim of the investigation was to assess ozone therapy capabilities in the correction of nonspecific antimicrobial resistance in infantile atopic dermatitis.

Materials and Methods. We observed 65 children (40 boys and 25 girls) aged from 8 months to 3 years with atopic dermatitis (an infantile form of the disease in accordance with the working classification presented in the scientific practical program “Atopic dermatitis in children: diagnosis, treatment and prevention”, Moscow, 2004). All the patients were diagnosed with a common medium-severe form of atopic dermatitis.

The study was conducted in accordance with the Declaration of Helsinki (adopted in June, 1964 (Helsinki, Finland) and revised in October, 2000 (Edinburgh, Scotland)) and approved by the Ethics Committee of the Kirov State Medical Academy. The patients’ parents gave a written informed consent in accordance with the Federal Law “Fundamentals of the legislation on the protection of citizens’ health”, July 22, 1993, No.54871.

The observed patients with atopic dermatitis were divided into two groups depending on the given

For contacts: Illek Yan Yurievich, e-mail: yanillek@gmail.com

treatment. Group 1 patients (n=33) were administered complex conventional therapy. The patients' parents were given recommendations on creating hypoallergenic conditions of life, the patients were administered individual hypoallergenic diet, medical and cosmetic skin care with the three-active bathing emulsion Emolium II during daily bathing, and three-active moisturizer Emolium II after bathing, creaming the injured areas with the skin cream Advantan (once a day for 7–10 days), Zyrtec intake (for 2 weeks), courses of Hylak forte, Linex and Kreon, vitamins A, E, B_5, B_6 , B_{15}.

Group 2 patients with atopic dermatitis (n=32) were administered the same complex treatment, but in combination with two courses of ozone therapy. Ozone therapy included oiling all the affected skin areas with ozonated olive oil (twice a day for 15 days) and the use of rectal insufflation with ozone-oxygen mixture given every other day (a total of 8 procedures). Ozone was produced with the use of the synthesizer (A-s-GOKSf-5-05-OZON, Electric Machine Building Works "LEPSE", Kirov) in which ozone is produced with the action of silent discharge on oxygen. Olive oil for external use was ozonized to the ozone outlet concentration of 20 mg/ml; the barbotage time of 100 ml of oil being 15 min [5]. At the specified ozone concentration its therapeutic dose is 75 μg per 1 kg of body mass. The volume of ozone-oxygen mixture for one rectal insufflation was calculated with the formula: [*body mass (kg)*×75]/20. The average volume of ozone-oxygen mixture for one rectal insufflation was 30–40 ml, the average volume of ozone-oxygen mixture for one course was 240–320 ml. Rectal insufflations of ozone-oxygen mixture were performed with the Janet syringe and a PVC tube with a tip attached to it, the patient lying on his left side with his legs bent. Before the procedure, the plastic tip was treated with vaseline and placed into the patient's rectum at the depth of 2 cm, then the needed volume of ozone-oxigen mixture was injected by pressing slowly the plunger of the Janet syringe. The entire procedure takes 1.5–2 min.

The first course of ozone therapy began on day 1–2 of the observation, the second course was given 3 months after the beginning of observation. No complications or adverse reactions in the patients undergoing complex treatment in combination with ozone therapy were observed.

The clinical parameters were studied in all the observed children, the onset and duration of clinical remission were defined, inoculations from the affected areas of the skin to the microflora and staphylococcus identification were performed.

To assess non-specific antimicrobial resistance (NAMR), the content of anti-α-staphylolysin in blood serum was defined in the first 1–2 days of observation (exacerbation period) and in 23–28 days after the beginning of the treatment (clinical remission period), the indices of phagocytic activity of neutrophils (PAN), the phagocytic index (PI) and test of nitro blue tetrazolium reduction (NBT-test) in the cytoplasm of neutrophils were analyzed, pattern-recognising receptors — toll-like receptors (TLR-2 and TLR-6) — were studied. The NAMR indices in patients with atopic dermatitis were compared with those in 80 healthy children of the same age from the city of Kirov and Kirov region.

Inoculations from the affected areas of the skin and staphylococcus identification in the patients with atopic dermatitis were performed on vitelline-salt agar according to the Guidelines of the USSR Ministry of Health (order No.534 from 22.04.1985) on the application of standardized microbiological (bacteriological) research methods in the clinical diagnostic laboratories of medical and profilactic facilities. The content of anti-α-staphylolysin in blood serum was determined in neutralization reaction with the use of staphylococcal α-toxin, a sample of standard rabbit anti-α-staphylolysin and erythrocytes (Rezepov F.F. et al., 1985); the results were expressed in IU/ml. PAN was assessed using latex particle with the size of 1.1 microns as a phagocytized object (Sigma, USA) by S.G. Potapova et al. (1977); the results were expressed in percentage terms. PI was calculated as the average number of latex particles absorbed by one neutrophil. In NBT-test setting, neutrophils activation was performed with latex, the number of cells, forming diphormazane granules was counted (Petrov R.V. et al., 1992); the results were expressed in percentage terms. The investigation of toll-like receptors 2 and toll-like receptors 6 was conducted at the flow cytoflourimeter (Spics XL, Beckman Coulter Inc., USA), assessing the expression of CD282 and CD286 markers (Bio-Chem Mak Diagnostics, Moscow, Russia) on lymphocytes, monocytes and neutrophils in percentage terms and density — in conventional units.

The data obtained in the study of NAMR indices in patients with atopic dermatitis, were processed by the method of variational statistics with the assessment of the arithmetic mean (M), standard deviation (δ) and the mean square error (m), the coefficient of reliability of the differences between the compared values (p) using the Student–Fisher table. Processing of the digital data was performed by the Microsoft Office Excel Mac 2011 application.

Results and Discussion. The studies have shown that 69% of the patients with the infantile form of common medium-severe atopic dermatitis were found to have their skin colonized with staphylococcus. In inoculations from the affected skin areas in these patients during exacerbation of the disease, staphylococcus aureus (44%) was most often inoculated, Staphylococcus epidermidis (27%), Staphylococcus saprofites (19%) or Staphylococcus aureus + Staphylococcus saprofites (10%) microbial associations were inoculated less frequently.

Both the groups of patients with atopic dermatitis during the exacerbation of the disease (Table 1) showed

an increase in the content of anti-α-staphylolysin in blood serum (p<0.01; p<0.02), a decrease in the phagocytic activity of neutrophils (p<0.001; p<0.001), phagocytic index values (p<0.001, p<0.001) and NBT-test (p<0.02, p<0.001).

No statistically significant difference between the changes in NAMR indices in groups 1 and 2 of the patients with atopic dermatitis was found. There were no significant changes in the expression of toll-like receptors 2 and toll-like receptors 6 on lymphocytes, monocytes and neutrophils in the both groups of patients with atopic dermatitis during the exacerbation of the disease, either (Table 2).

It was established that complex therapy as well as complex treatment in combination with ozone therapy in the respective groups of patients with infantile medium-severe forms of atopic dermatitis lead to the improvement of the general state and appetite, sleep normalization, reduction and disappearance of skin itching and other clinical manifestations of the disease.

However, the onset of full clinical remission in patients with atopic dermatitis treated with complex conventional therapy was stated in 25.1±1.1 days, and in those receiving complex treatment in combination with ozone therapy it began in 19.6±0.9 days after the beginning of the treatment. Consequently, the onset of clinical remission in the group of patients treated with ozone therapy as part of complex treatment was registered 5.5 days earlier on average (p<0.001).

In the period of clinical remission the patients with atopic dermatitis, receiving complex conventional therapy (See Table 1) were found to maintain elevated levels of anti-α-staphylolysin (p<0.01) in blood serum, a decrease in phagocytic activity in neutrophils (p<0.001), the phagocytic index (p<0.001) and NBT-test (p<0.05). At the same time these patients showed the signs of activation of toll-like receptors during clinical remission (See Table 2), which manifested itself in an increase in the density of toll-like receptors 2 expression on the lymphocytes (p<0.001); an increase in the relative number of monocytes (p<0.001) and neutrophils (p<0.01), expressing toll-like receptors 2; an increase in the relative number of neutrophils (p<0.01), expressing toll-like receptors 6.

Table 1

The content of anti-α-staphylolysin in blood serum and phagocytosis indices in healthy children and in patients with atopic dermatitis treated with complex conventional therapy (group 1) and complex therapy in combination with ozone therapy (group 2) M±m

Indices	Healthy children (n=80)	Period of disease exacerbation		Period of clinical remission	
		Group 1 (n=33)	Group 2 (n=32)	Group 1 (n=33)	Group 2 (n=32)
Anti-α-staphyilolysin, IU / ml	1.20±0.17	2.02±0.25*	1.99±0.28*	1.98±0.23*	1.46±0.20
PAN, %	73.40±1.66	57.84±0.33*	60.21±2.75*	64.11±2.58*	71.38±1.45
PI	11.80±0.29	9.25±0.38*	8.78±0.47*	8.98±0.25*	11.58±0.36
NBT-test, %	17.60±1.03	14.25±0.28*	13.25±0.62*	14.72±0.72*	18.23±0.52

* — p<0.05–0.001 in comparison of the values with the indices in virtually healthy children.

Table 2

Indices of TLR-2 and TLR-6 expression in healthy children and in patients with atopic dermatitis treated with complex conventional therapy (group 1) and complex therapy in combination with ozone therapy (group 2) (M±m)

Indices	Healthy children (n=80)	Period of disease exacerbation		Period of clinical remission	
		Group 1 (n=33)	Group 2 (n=32)	Group 1 (n=33)	Group 2 (n=32)
TLR-2 expression on:					
lymphocytes, %	0.11±0.01	0.11±0.01	0.14±0.02	0.20±0.08	0.29±0.09*
lymphocytes, pl., conv.u.	1.67±0.06	1.82±0.18	1.69±0.16	3.00±0.29*	3.02±0.27*
monocytes, %	34.14±2.41	36.88±2.97	33.38±2.78	50.86±3.27*	57.43±2.54*
monocytes, pl., conv.u.	1.43±0.03	1.50±0.07	1.31±0.04	1.54±0.10	1.45±0.12
neutrophiles, %	0.25±0.02	0.38±0.08	0.30±0.08	0.98±0.13*	1.41±0.17*
neutrophiles, pl., conv.u.	1.96±0.15	2.14±0.18	2.28±0.10	2.02±0.17	2.09±0.14
TLR-6 expression:					
lymphocytes, %	0.15±0.01	0.14±0.03	0.18±0.02	0.11±0.02	0.17±0.01
lymphocytes, pl., conv.u.	2.37±0.07	2.43±0.24	2.11±0.12	1.98±0.21	3.45±0.20*
monocytes, %	0.19±0.03	0.26±0.05	0.24±0.02	0.28±0.05	0.45±0.04*
monocytes, pl., conv.u.	2.32±0.06	2.28±0.16	2.18±0.25	2.39±0.21	3.31±0.27*
neutrophils, %	0.18±0.01	0.19±0.14	0.16±0.01	0.30±0.04*	0.39±0.03*
neutrophils, pl., conv.u.	1.73±0.08	1.87±0.21	1.88±0.13	2.07±0.26	3.49±0.26*

* — p<0.05–0.001 in comparison of the values with the indices in virtually healthy children.

In patients with atopic dermatitis who received complex treatment in combination with ozone therapy, in the period of clinical remission the content of anti-staphilolysin in blood serum, the index of phagocytic activity of neutrophils, the values of phagocytic index and NBT-test were not significantly different from those in the indices of virtually healthy children.

At the same time, this group of patients in the period of clinical remission showed the signs of high functional activity of pattern-recognizing receptors manifesting themselves in an increase in the density of toll-like receptor 2 expression on lymphocytes ($p<0.001$); an increase in the relative number of lymphocytes ($p<0.05$), monocytes ($p<0.001$) and neutrophils ($p<0.001$), expressing toll-like receptors 2; an increase in the expression density of toll-like receptors 6 on lymphocytes ($p<0,001$), monocytes ($p<0,01$) and neutrophils ($p<0,001$); an increase in the relative number of monocytes ($p<0.001$) and neutrophils ($p<0.001$), expressing toll-like receptors 6.

The obtained data indicate a pronounced activation of toll-like receptors, which play a key role in the detection of pathogenic microorganisms invading the barrier tissues in humans and early implementation of the mechanisms of congenital immunity.

The children with atopic dermatitis treated with complex conventional therapy were found to have the signs of exacerbation of the disease 4.3±0.2 months after the beginning of clinical remission. The patients, who, together with the complex conventional treatment underwent two courses of ozone therapy with 3 month intervals between them, did not show clinical signs of disease exacerbation for 14.5±0.3 months. Thus, the duration of clinical remission in group 2 patients is more than three times as much ($p<0.001$) in comparison with that in group 1 patients.

Conclusion. Patients with an infantile form of common medium-severe atopic dermatitis during the exacerbation of the disease are found to have their skin colonized with staphylococcus and pronounced changes in the indices of non-specific antimicrobial resistance. In children treated with complex conventional therapy, clinical remission is of a brief character with retention of changes in the indices of nonspecific antimicrobial resistance. Inclusion of ozone therapy in the complex treatment of patients with atopic dermatitis results in activation of toll-like receptors 2 and toll-like receptors 6, normalization of antistaphyilolysin in blood serum and phagocytosis indices, and a pronounced increase in the duration of clinical remission.

Research Funding and Conflict of Interest. The study was not funded by any sources, and the author declares to have no conflict of interest related to this study.

References

1. Illek Ya.Yu., Zaytseva G.A., Galanina A.V. *Atopicheskiy dermatit u detey rannego vozrasta* [Atopic dermatitis in young children]. Kirov; 2007.

2. Snarskaya E.S. Some clinico-immunological aspects of the pathogenesis of atopic dermatitis and the role of toll-such receptors. *Lechashchiy vrach* 2012; 4: 29–33.

3. Lin Y.T., Yang Y.H., Hwang Y.W., et al. Comparison of serum specific IgE antibodies to staphylococcal enterotoxins between atopic children with and without atopic dermatitis. *Allergy* 2000; 55(7): 641–646, http://dx.doi.org/10.1034/j.1398-9995.2000.00523.x.

4. Matsui K., Nisnikava A., Suto H., et al. Comparative study of Staphylococcus aureus isolated from lesional and non-lesional skin of atopic dermatitis patients. *Microbiol Immunol* 2000; 44(11): 945–947.

5. Maslennikov O.V., Kontorshchikova K.N., Shakhov B.E. *Rukovodstvo po ozonoterapii* [Guidelines on ozone therapy]. Nizhny Novgorod; 2012.

6. Kosheleva I.V., Ivanov O.L. Ozonoterapiya v kompleksnom lechenii ekzemy. V kn.: *Materialy 3-y nauchno-prakticheskoy konferentsii "Ozon i metody efferentnoy terapii v meditsine"* [Ozone therapy in complex treatment of eczema. In: Proceedings of the 3rd research and practice conference "Ozone and efferent therapy methods in medicine"]. Nizhny Novgorod; 2000; p. 109–110.

7. Kosheleva I.V., Ivanov O.L., Vissarionov V.V. *Primenenie kislorodno-ozonovoy smesi v kosmetologii. Metodicheskie rekomendatsii No.2003/84* [The use of oxygen-ozone mixture in cosmetology. Guidelines No.2003/84]. Moscow; 2004.

8. Illek Ya.Yu., Galanina A.V., Suslova E.V., Mishchenko I.Yu., Leushina N.P., Tarasova E.Yu., Suslov I.N. Therapeutic effect of ozone in infantile atopic dermatitis. *Allergologiya i immunologiya* 2013; 14(2): 98.

9. Illek Ya.Yu., Suslova E.V., Gaynanova A.M., Kuznetsova V.V., Galanina A.V., Leushina N.P., Mishchenko I.Yu., Sizova O.G., Tarasova E.Yu. The effect of ozone therapy on clinical and immunological indices in atopic dermatitis in adolescents. *Permskiy meditsinskiy zhurnal* 2013; 30(3): 46–52.

10. Illek Y.Y., Zaytseva G.A., Galanina A.V., Vasilieva Y.A., Rybakova T.N., Suslova E.V. Immunomodulating and anti-relapse effects of ozone therapy in atopic dermatitis in preschool and primary school children. *Sovremennye tehnologii v medicine* 2013; 5(2): 89–92.

11. Suslova E.V., Illek Ya.Yu., Vasilieva Yu.A., Suslov I.N., Chaganov I.B. The immune disorders and their correction with ozone in case of children form of severe atopic dermatitis. *Medicinskij al'manah* 2013; 3(27): 171–172.