ULTRALOW DOSES

The Use of Anaferon (Pediatric Formulation) for Prophylaxis of Acute Respiratory Viral Infections in Preschool Children

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Anaferon (pediatric formulation) reduces the incidence of acute respiratory viral infections in sickly children and children with bronchial asthma and has a positive effect on the course of asthma. The preparation produces an immunomodulating effect (increases initially low IFN-γ levels and normalizes elevated levels of IL-1β), stimulates synthesis of IgA and IgG, exhibits cytoprotective activity, and improves local immunity of the upper airways in sickly children.

Key Words: *anaferon (pediatric formulation); bronchial asthma; acute respiratory viral infections; immunity*

The prevalence of acute respiratory viral infections (ARVI) in children is still high; more than 75% of these cases are recorded among sickly children [7]. Children with bronchial asthma (BA) deserve special attention, because 70-90% exacerbations of the disease are related to ARVI episodes. The production of IFN is low in sickly children and children with BA [2], which dictates the necessity of using preparations modulating the immune response as the prophylactic means. Therefore, studies of a new class of drugs, inductors of endogenous IFN, are an urgent problem. Anaferon (pediatric formulation, AP) belongs to this class of preparations.

AP contains antibodies to IFN-γ in ultralow doses. The immunomodulating and antiviral effects of AP are related to stimulation of the synthesis of early IFN (IFN-α and IFN-β) and late IFN-γ.

Here we determined the efficiency of AP for the prophylactics of ARVI in sickly children and children with BA attending child welfare institutions during the periods of unfavorable epidemic situations.

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MATERIALS AND METHODS

The study included 106 preschool children, of them 40 sickly children (kindergarten No. 81, Tomsk) and 66 children with BA (kindergarten No. 20, Seversk).

Sickly children were randomly divided into two groups. The main group 1 comprised 20 children receiving AP as the prophylactic means for 3 months (1 tablet daily, sublingually, independent of the meal). The control group 2 included 20 age-matched children receiving reference preparation Revit, a polyvitamin complex (1 tablet daily for 3 months).

The inclusion criteria for groups 1 and 2 were: age 2-4 years; the absence of individual hypersensitivity to preparation components, and signed informed consent of parents.

The children with BA were randomly divided into two groups. Children of the main group 3 (n=40) re-

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ceived AP for 3 months (1 tablet daily, sublingually, regardless the meal). The control group 4 comprised 26 children receiving placebo according to the same scheme.

The inclusion criteria for groups 3 and 4 were: age 2-7 years, mild or moderate BA, and signed informed consent of parents.

The formed groups were matched by the sex, age, and health status.

In case of ARVI, symptomatic means were applied in all groups, when needed. In groups 1 and 3, the therapeutic scheme of AP treatment was used in these cases: 1 tablet every 30 min during the first 2 hours (day 1), then 1 tablet 3 times a day sublingually independent of the meal (days 2-5). Children of group 4 received placebo according to the same scheme, children of group 2 received only symptomatic therapy. After convalescence, the child received the preparation according to the prophylactic scheme.

The following methods and parameters were used: clinical and anamnestic method with calculation of the index of epidemiological efficiency (IEE) and coefficient of epidemiological efficiency (CEE) [6], cytological analysis of impression smears from the nasal mucosa [5], IFA for evaluation of total serum IgE (standard Dr. Fooke kits), IL-1β, IL-4, IFN-γ (kits from Proteinovyi Kontur Company), measurement of lysozyme activity [1], content of secretory IgA (sIgA) in nasal washout fluid, and serum content of IgA and IgG [8], evaluation of the function of external respiration (Jaeger).

The data were processed statistically using SAS 8, SPSS v 11.5 software. Quantitative data corresponding to normal distribution were presented using descriptive statistic values (median Me, 25% quartile Q1, 75% quartile Q3) and standart deviation. The correspondence of the experimental sample to normal distribution was verified using Shapiro—Wilk test. Qualitative data were described using absolute and relative frequency characteristics. Significance of differences between independent samples was evaluated using contingency tables and exact Fisher test [4].

RESULTS

Evaluation of IEE and CEE in the group of sickly children and children with BA showed better protection from ARVI in children receiving AP compared to the control. In group 1, IEE was 2.06 and CEE was 52%. In group 3, IEE was 1.6 and CEE was 38.1%.

In group 1, 45% children had no respiratory infections, while in group all patients had ARVI ($p<0.05$). Single ARVI was recorded in 30% patients of group 1 and in 30% patients in group 2. Two and more ARVI

TABLE 1. Parameters of Morphofunctional State of Nasal Mucosa in Sickly Children ($M\pm m$)

Parameter	Group 1			Group 2		
	before therapy	after 3 months	after 6 months	before therapy	after 3 months	after 6 months
Neutrophils						
%	22.20±2.61	12.05±1.87*	13.36±1.16*	19.77±2.04	23.81±2.05	19.59±1.88
index of destruction	1.23±0.32	0.54±0.19**	0.56±0.21**	1.25±0.28	1.22±0.22	1.22±0.23
index of cytolysis	0	0	0	0	0	0
Squamous epithelium						
%	38.65±3.71	43.97±2.65**	42.44±2.34**	40.50±1.01	38.22±1.61	42.84±1.20
index of destruction	1.68±0.35	0.99±0.21**	1.52±0.27	1.59±0.17	2.01±0.23	1.64±0.22
index of cytolysis	0.050±0.001	0	0.020±0.001**	0.070±0.015	0.060±0.002	0.050±0.001
Cylindrical epithelium						
%	35.78±2.97	41.65±3.54**	41.56±3.26**	36.34±1.05	34.86±0.99	34.90±1.40
index of destruction	1.89±0.36	1.05±0.27**	1.14±0.31**	1.74±0.15	1.92±0.21	1.39±0.18
index of cytolysis	0.130±0.004	0	0.090±0.003**	0.050±0.002	0.050±0.005	0.06±0.003

Note. *$p<0.01$, **$p<0.05$ compared to values before therapy.

TABLE 2. Dynamics of Parameters of Destruction of Nasal Mucosa Cells in Children with BA (Me (Q1-Q3))

Parameter	Observation term	Group 3	Group 4
Mean index of destruction			
squamous epithelium	Before therapy	0.55 (0.20-0.67)	0.53 (0.23-0.85)
	After 1 months	0.20** (0.00-0.56)	0.88 (0.16-1.57)
	After 3 months	0.26* (0.00–0.61)	0.75 (0.20-1.12)
cylindrical epithelium	Before therapy	0.26 (0.00-0.55)	0.28 (0.13-0.57)
	After 1 month	0.15** (0.06-0.30)	0.51 (0.15-1.00)
	After 3 months	0.19 (0.06-0.42)	0.31 (0.10-0.51)
neutrophils	Before therapy	1.15 (0.73-1.60)	1.23 (0.68-2.31)
	After 1 month	0.43*++ (0.18-0.70)	2.04+ (1.29-2.67)
	After 3 months	0.53*++ (0.33-1.11)	1.22 (0.69-2.08)
Index of neutrophil destruction	Before therapy	0.06 (0.00-0.15)	0.06 (0.00-0.19)
	After 1 month	0.00*++ (0.00-0.00)	0.22 (0.00-0.30)
	After 3 months	0.00*++ (0.00-0.00)	0.08 (0.00-0.33)

Note. *$p<0.05$, **$p<0.001$ compared to group 4; +$p<0.05$, ++$p<0.001$ compared to values before therapy.

episodes were recorded in 25 and 70% children of groups 1 and 2, respectively ($p<0.05$).

In group 3 and 4, 50 and 19.2% children had no cases of ARVI, 45 and 27% had single ARVI episode, and 5 and 53.8% children had two ARVI episodes ($p<0.001$).

The mean duration of the disease was 7.8±2.6 and 12.5±2.9 days in groups 1 and 2, respectively ($p<0.05$). The duration of ARVI in children with BA receiving AP and placebo was 7.5±0.9 and 19.1±2.6, respectively ($p<0.001$). All children well tolerated the preparation.

The study showed that during the first 3 months of observation, prophylactic treatment with AP significantly decreased the number of BA exacerbations compared to the placebo group ($p<0.001$). No significant differences between the groups by the number of BA exacerbations were revealed from the 4th to the 6th month of observation. The mean duration of BA exacerbation in group 3 during the first 3 months and from the 4th to the 6th month was significantly lower ($p<0.05$) than in group 4. The need in β_2-adrenoceptor agonists significantly decreased in group 3 compared to group 4 during the first 3 months of the study ($p<0.05$).

Our study showed that treatment with AP produced an indirect positive effect on the peak expiratory flow rate (this parameters considerably increased by the end of the 3rd month of observation compared to the corresponding parameters in the placebo group, $p<0.05$).

Comparison of the mean IFN-γ, IL1-β, and IL-4 levels in groups of children with BA before and after treatment revealed no significant differences. Analysis of the dynamics of parameters corresponding to high and low levels of these cytokines showed the following significant changes. In group 3 children with initially low level of IFN-γ, the increase in this parameter by the end of the 3rd month was significant ($p<0.05$) in both intra- and intergroup comparisons. In group 3 children with initially high concentration of IL-1β this parameter significantly decreased by the end of the 3rd month against the background of treatment. Intergroup comparison revealed only insignificant decrease of elevated IL-1β content. The content of IL-4 in all groups changed insignificantly throughout the observation period.

The study of morphofunctional changes in nasal mucosa showed that treatment with AP had a positive effect on local cell defense factors (Tables 1, 2). We observed normalization of the epithelium against the background of AP treatment in groups 1 and 3, which manifested in significantly increased content of cylindrical and squamous epithelium and a decrease in the mean parameters of destruction and index of cytolysis of cylindrical and squamous epithelial cells (Tables 1, 2). These changes improved mucociliary transport [5]. The content of neutrophils on the surface of nasal mucosa significantly decreased, their structure was preserved. The lymphocyte count on the nasal mucosa remained practically unchanged throughout the observation period.

The revealed quantitative and qualitative changes in cell composition of the nasal mucosa in children of the main group persisted until the next examination after 3 months. It can be assumed that this effect is determined by the effect of endogenous INF on cell

TABLE 3. Parameters of Local and Systemic Immunity in Sickly Children (*M*±*m*)

Parameter	Group 1			Group 2		
	before therapy	after 3 months	after 6 months	before therapy	after 3 months	after 6 months
Lysozyme, %	45.79±3.27	73.85±3.12***	69.17±2.98**	46.11±2.86	42.04±3.09*++	54.88±3.13*+
sIgA, g/liter	0.294±0.030	0.430±0.020***	0.377±0.010***	0.295±0.020	0.410±0.010***+	0.302±0.01+++
IgA, g/liter	0.64±0.04	1.23±0.03**	—	0.68±0.06	0.67±0.06++	—
IgG, g/liter	7.10±0.30	12.40±0.70*	—	7.40±0.20	10.50±0.40*+	—

Note. *p<0.05, **p<0.01, ***p<0.001compared to values before treatment; +p<0.05, ++p<0.01, +++p<0.001 compared to group 1.

metabolism, which leads to recovery of the colloidal properties of the cytoplasm in epithelial cells and improved the integrity of the cell membranes. Moreover, the need in local phagocytic function of neutrophils decreases and, hence, their structure is preserved due to lysis of microbes [5]. In children of the control group, no reliable changes by all parameters of the nasocytogram within the group were noted at the same terms of the study (Table 1, 2).

Deficiency of local defense factors was revealed in sickly children during the first examination (Table 3). Lysozyme activity of nasal washout fluid in children of groups 1 and 2 was 45.79±3.27 and 46.11±2.86, respectively, *i.e.* considerably below the normal (76.20± 3.20%) [5]. After prophylactic course of AP, lysozyme activity in the main group considerably increased compared to the control group (p<0.001). After 3 months, lysozyme activity in nasal washout fluid tended to decrease, but remained above the initial level (p<0.01; Table 3). In contrast to group 1 children, lysozyme activity in nasal washout fluid decreased (p<0.05) during unfavorable epidemiological situation (February-March)

In all examined sickly children, the content of sIgA in nasal washout fluid was below the normal [3]. Treatment with AP increased sIgA content in nasal washout fluid by the end of the prophylactic course (p<0.001). High level of sIgA in nasal washout fluid persisted for 3 months after the end of the prophylactic course (p<0.001). In group 2 children, the concentration of sIgA also significantly increased (p<0.001). Taking into account the absence of nonspecific prophylactics and high morbidity in this group, this dynamics can be explained by more pronounced antigen load to the nasal mucosa. During catamnestic observation, the level of sIgA in the control group also progressively decreased below the normal (Table 3). Intergroup comparison revealed a significant increase in sIgA content in nasal washout fluid in group 1 children compared to group 2 children 3 and 6 months after the start of observation (p<0.05 and p<0.001, respectively).

The initial levels of serum IgA and IgG in children of groups 1 and 2 were similar (Table 3) and corresponded to the lower boundary of the normal [3]. Treatment with AP increased serum content of IgA and IgG in group 1 children (p<0.01 and p<0.02, respectively).

Thus, treatment with AP is effective and safe method of ARVI prophylactic in sickly preschool children and children with BA. The prophylactic effect of AP persisted for 6 months. AP produces a positive effect on the course of mild and medium-severe BA.

AP produces a pronounced cytoprotective effect, which manifested in improvement of morphofunctional characteristics of cylindrical and squamous epithelium of the nasal mucosa. Moreover, AP produces a positive effect on the state of local immunity of the upper airways due to the increase in lysozyme activity and sIgA content in nasal washout fluid in sickly children. The preparation produces an immunomodulating effect on the content of IFN-γ and IL-1β in children with BA and an immunostimulating effect on the content of IgA and IgG in sickly children

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