Clinical and Immunological Efficiency of Anaferon (Pediatric Formulation) in Calicivirus Infection in Children

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Therapy with anaferon (pediatric formulation) during the acute period of calicivirus infection shortened the duration of the main symptoms of the disease and period of virus release. Changes in the immunological status included increased production of IgA and IgM and activation of IFN-α synthesis.

Key Words: *acute intestinal infection; caliciviruses; children; anaferon (pediatric formulation)*

Acute intestinal infections (AII) remain a crucial problem of pediatrics and are important for medical science and practice. Virus diarrhea plays the leading role in AII [7,8]. The application of new molecular and genetic diagnostic methods has shown caliciviruses to be the most common (after rotaviruses) cause of acute gastroenteritis outbreaks (up to 36-40%) in industrial countries [10-12]. Specific antibodies to caliciviruses are present in the serum of 58-70% adult individuals.

Despite high prevalence and frequency of viral AII in children, many problems remain unsolved, including the problems of therapy of these patients. We found only few reports of foreign and Russian investigators about antiviral therapy of AII in children. Most of these reports are devoted to the treatment of rotaviral gastroenteritis [1,3,9]. The leading role in antiviral protection is played by the IFN system. IFN, cytokines exhibiting unique antiviral properties, can suppress replication of many RNA and RNA-containing viruses via inhibition of transcription and translation of viral matrices [2,4,13]. At the same time, the immune and cytokine status in children with calicivirus infection (CVI) was never evaluated.

Here we studied clinical and immunological efficiency and safety of anaferon (pediatric formulation, AP) as the means for etiotropic therapy of acute gastroenteritis caused by CVI in children.

MATERIALS AND METHODS

The efficiency of AP was evaluated by analyzing clinical and laboratory data of 60 children (age 1-15 years) with CVI-induced AII, patients of Hospital of Intestinal Infections, Institute of Children Infections, Federal Agency for Health Care and Social Development. Laboratory tests included clinical analyses of the blood and urine, coprocytogram, analysis of feces for pathogenic and opportunistic microflora, and IEA of feces for the presence of rotavirus antigen. The presence of rotavirus in feces was detected using transmission electron microscopy allowing agent identification by morphological signs (Fig. 1).

Immunophenotyping of peripheral blood lymphocytes was carried out in the lymphocytotoxicity test

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using a panel of monoclonal antibodies (series IKO, Medbiospektr Research-and-Production Center) [5]. The concentration of immunoglobulins IgA, IgM, and IgG was routinely measured by the method of radial immunodiffusion with monospecific antisera (Bacterial Preparation Plant, Institute of Epidemiology and Microbiology, Nizhnii Novgorod). The total IgE content was measured by enzyme-linked immunosorbent assay using commercial test-systems (Polygnost Company). The total pool of circulating immune complexes was performed by polyethyleneglycol precipitation on plates. The levels of cytokines (TNF-α, IFN-γ, IL-4, and IL-6) in blood sera were measures by enzyme-linked immunosorbent assay (Proteinovyi Kontur) according to manufacturer's instructions. Proliferative activity of T cells was evaluated by the reaction of lymphocyte blast-transformation induced by phytohemagglutinin (Difco) in a final concentration of 10 μg/ml in cultures of the whole blood with morphological analysis of the results [6].

The children were randomly divided into two groups. The main group (n=30) received AP according to therapeutic scheme: 1 tablet (0.3 g) every 30 min over the first 2 hours and then 3 more tablets (0.3 g) with equal time intervals during day 1 and 1 tablet (0.3 g) 3 times a day during the subsequent 4 days. Controls (n=30) received placebo according to the same scheme.

All patients received pathogenetic (basis) therapy: dietotherapy, rehydratation, enterosorption, administration of pre- and probiotics, and enzymes. The children received infusions of glucose and saline in a volume corresponding to physiological requirements and pathological losses. In the group of patients receiving AP, infusion therapy was performed in 63.3% children of the AP group (1.42±0.16 days) and in 43.3% patients of the reference group (1.61±0.21 days). In the course of dynamic observation, the severity and duration of the main symptoms (intoxication, fever, vomiting, diarrhea, catarrhal symptoms) and duration of virus shedding were evaluated.

RESULTS

Clinical and immunological efficiency of treatment was evaluated in patients after completion of the course (n=60). The mean age of patients included in the study was 5.9±0.6 years; children below 3 years predominated (41.7%), children aging 3-7 years and above 7 years constituted 25 and 33.3%, respectively. No gender differences were revealed; 40% examinees were admitted to the hospital on day 1 of the disease, 50% on day 2, and 10% of day 3.

The incidence of various clinical symptoms of AII (general infection symptoms and gastrointestinal symptoms) was similar in the treatment and placebo group. The children primarily had medium-severe form of the disease (70%), severe and mild forms of the disease were diagnosed in 16.6 and 13.3% patients, respectively. All patients complained of repeated vomiting. Flabbiness and malaise were noted in 95% patients, 16.7% had anxiety, and 11.7% had headache; fever was observed in all patients.

Acute period (2.7±0.1 days) of CVI was characterized by considerable shifts in the immune status of patients compared to healthy children. *In vivo* synthesis of antiviral cytokines IFN-α and IFN-γ and antiinflammatiory cytokine IL-10 was most markedly activated in this infection. The level of circulating IFN-α, IFN-γ, and IL-1β somewhat increased, while the content of TNF-α and IL-10 decreased with increasing the severity of the disease. Production of Th2 cytokine IL-4 did not reach the normal level, which confirmed primarily cellular Th1-dependent profile of the immune defense.

In children receiving AP, the duration of the disease and its main symptoms was shorter than in patients receiving placebo (Fig. 2). In children receiving AP therapy, symptoms of intoxication and fever disappeared more rapidly (p<0.001), vomiting and diarrhea stopped earlier by one day than in the placebo group. Other symptoms (nausea, stomachache, meteorism, exicosis) also disappeared at earlier terms than in the group receiving placebo. Addition of AP to the complex therapy shortened the duration of the main clini-

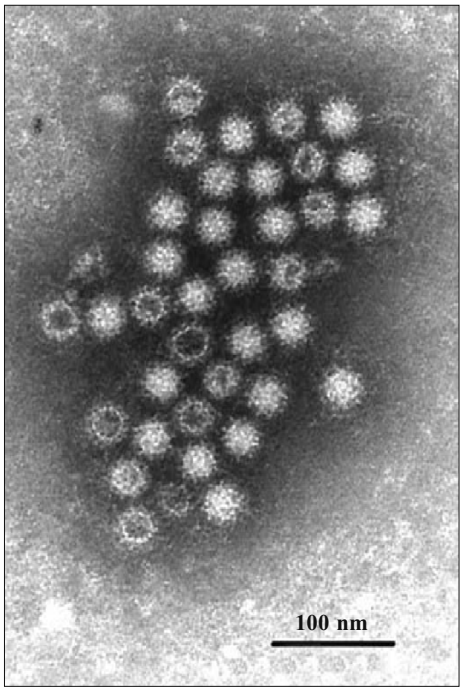

Fig. 1. Novovirus. Electron microscopy.

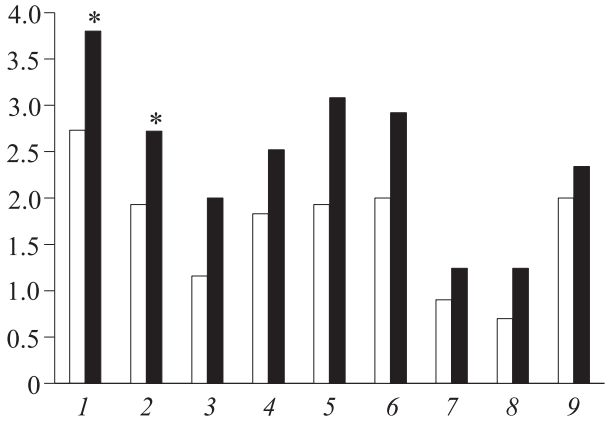

Fig. 2. Clinical efficiency of AP in CVI. Open bars: AP; dark bars: placebo. *1*) duration of intoxication; *2*) fever; *3*) nausea; *4*) vomiting; *5*) anorexia; *6*) diarrhea; *7*) exicosis; *8*) stomachache; *9*) meteorism. **p*<0.001 compared to healthy children.

cal manifestations of calicivirus gastroenteritis by on average 2 days (2.4±0.2 and 4.2±0.2 days in the treatment and control groups, respectively, $p<0.05$).

The most drastic changes were observed in the duration of virus shedding. In children receiving AP, the duration of virus shedding decreased by 2 times compared to patients receiving placebo (5.70±0.47 days *vs*. 9.80±0.58 days). During convalescence, norovirus shedding was observed in 3.33% patients treated with AP and 53.33% children receiving placebo.

Evaluation of the immune status and cytokine response in the compared groups also revealed significant differences. In children treated with AP we observed an increase in the number of $CD3^+$ (53.83±1.75 *vs.* 44.77±2.21%, $p<0.05$), $CD4^+$ (28.1±1.11 *vs.* 25.63±1.91%), $CD20^+$ (20.37±1.38 *vs.* 17.93±1.38%) and activation of production of IgA (0.53±0.05 *vs.* 0.380±0.053 g/liter, $p<0.05$) and IgM (0.970±0.076 *vs.* 0.83±0.06 g/liter) by the period of early convalescence compared to acute phase of the infection. TNF-α synthesis was also activated (13.02±1.41 *vs.* 10.83±1.28 pg/ml). At the same time, we observed no tendency to a decrease in the content of proinflammatory cytokines IL-1β (106.46±22.52 *vs.* 27.3±6.8 pg/ml in healthy children, $p<0.01$), TNF-α (83.25±26.92 *vs.* 19±4 pg/ml in healthy children, $p<0.01$), while the level of IFN-γ corresponded to that in healthy individuals (90.40±13.16 *vs.* 92±24 pg/ml).

In patients receiving placebo, no increase in the number of cells carrying CD3 receptors was observed (49.70±1.99 *vs.* 49.57±2.00%); by the period of convalescence, the number of $CD16^+$-cells (natural killers) did not exceed the normal values (19.50±1.43% *vs*. 15.5±1.8% in healthy individuals), production of IgA also did not increase (0.59±0.06 *vs*. 0.55±0.06 g/liter); at the same time, synthesis of IgM tended to increase (0.70±0.05 *vs*. 0.586±0.049 g/liter). In children receiving placebo, the level of IFN-γ by the period of convalescence was lower than in patients treated with AP and even lower than in healthy children (56.80±9.49 *vs*. 90.40±13.16 and *vs.* 92±24 pg/ml, $p<0.05$ и $p>0.05$, respectively), which probably attests to insufficient activation of cell-mediated immune response in children of this group.

Analysis of cases of viral gastroenteritis showed that CVI is characterized by peculiar clinical symptoms (prevalence of vomiting) and peculiar changes in the immune status of patients. Addition of AP to complex treatment as a means of etiotropic therapy is substantiated. The positive effects of AP on the duration of the main symptoms, immune response of the organism, and virus shedding are proven. Rapid clearance of the virus from the organism has high epidemiological efficiency, because long-term excretion of the virus promotes its circulation and increases the levels of sporadic and outbreak morbidity.

TABLE 1. Cytokine Concentration (pg/ml) in Serum of Children with CVI (Days 1-3 of the Disease; *M*±*m*)

Cytokine	Healthy children (*n*=10)	Form of the disease		
		mild (*n*=8)	medium-severe (*n*=42)	severe (*n*=10)
IFN-α	0.9±0.2	7.9±1.3*	10.0±1.3*	10.8±2.6*
IFN-γ	26±8	71.1±22.4	87.2±18.6	116.0±23.4*
IL-1β	111±45	52.3±9.4	95.8±26.8	124.4±88.0
TNF-α	70±23	62.0±18.5	56.3±19.1	9.8±9.5*
IL-6	22.0±9.7	28.3±3.2	17.8±1.6	22.1±3.4
IL-4	51±7	15.8±3.0*	9.7±1.6*	32.7±18.5
IL-10	4.8±2.8	183.2±73.0*	108.7±81.2	33.2±11.5*

Note. **p*<0.05 compared to healthy children.

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