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Immunotherapy in children with acute enteric infections Experience using new immunomodulator 'Gepon'

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The research into the therapeutic response to a new immunomodulator 'Gepon' in 30 patients with acute enteric infection established that its inclusion in combination therapy of moderately severe forms of Acute Enteric Infection (AEI) in children from the commencement of illness; appreciably raises clinical and sanitising efficacy of antibacterial therapy; reduces the duration of the acute period of the disease; positively influences quantitative and qualitative balance of intestinal microflora (disbiosis)

The protective role of immune system of a human body is well known. The ability of the body to resist activators of infection diseases at all stages of infectious process, including enteric infections with the initial adhesion and colonisation of intestinal epithelium, is determined by activity of specific and nonspecific factors of protection. Prior to the beginning of disease, the condition and adequacy of immune resistance in many respects determines not only the possibility of disease, but also its severity, the duration of the acute period, cyclicity, effectiveness of clearance of pathogens and outcome of the illness.

Numerous studies into immune status [1-4] have established, that there are both specific and nonspecific disturbances of resistance in children with enteric infections connected with initial (premorbid) condition combined with immune deficiency. To correct disfunction of immunity and to increase the nonspecific immune resistance in children with acute enteric infection, various preparations are used in clinical practice as specific agents such as immunoglobulin, containing antibodies against various pathogens of AEI, and nonspecific agents such as pentocsil, lysozyme, sodium nucleinate etc. which stimulate antibody development and tissue immunity mechanisms, repair processes and antibacterial activity [2,4-6]. Recombinant interferons such as viferon in suppositorys, reaferon in microclisters, entalferon in tablets, kipferon etc. have begun to be used over past few years. Activity of Natural Killer, T-helper, phagocytic activity, intensity of a B-lymphocytes differentiation increases with the use of these preparations, which inhibits and eliminates the pathogens of AEI, preventing a long disease duration and the associated complications [7,8]. Immunoprotective effects are established also with probiotics (such as bifidumbacterin, bifiform, enterol etc.) and biologically active food additives (BAD "favorit" etc.)

As a rule, the majority of preparations influence immune resistance and are used in clinical practice for special indications (adverse premorbid background, long course etc.) and mainly during the early incubation of disease. However, as numerous researches of immune status in AEI have shown, including those carried out in our clinic [9], in the initial stage (Day 2-3) of intestinal infection and in most cases (78 %), there are negative changes in essential parts of immune function, particularly cellular (T-helper) immunity. With the AEI bacterial aetiology (particularly with salmonellosis) the quantitative content of OKT3 was reduced to 34-38,5% (normal 64

± 7,5%), OKT4 reduced to 22-24,7% (normal 38,6 ± 8,7%), and the content of circulating immune cells in the blood serum increased to 0,12-0,18 optical density units (normal 0,075 ± 0,02). In dynamics, especially in combination therapy with broad spectrum antibiotics, there was a further decrease of these parameters (OKT3 down to 32,6 ± 0,4 %, OKT4 23,7± 1,2 %). The quantitative content of B – cells and Ig A, M, G classes in blood serum, irrespective of therapy, remained for 7-10 day of illness without any essential changes from their initial level at the beginning of the course of treatment. The use of oral immunoglobulins (antirotavirus Ig) with commensal microorganisms such as kefir "Bifidok", BAD "Favorite" in combination therapy of AEI in children for 7-10 day of treatment results in substantial improvement of cellular (T-helper) immunity, quantitative and qualitative structure of intestines microflora. The infectious toxicosis symptoms and the diarrheic syndrome disappeared faster in these patients and increases the sanitizing efficacy of antibacterial therapy.

The benefit from the inclusion of drugs with immunomodulative effect in the early days of disease in combination therapy of AEI in children has already been demonstrated. Among these drugs there is one noteworthy new domestic immunomodulator "Gepon", produced by OOO "Immapharma"(register. №0000yshch/ 04-2001). Gepon is the representative of the new class of immunoregulative peptide, synthetic homologues of hinged region ezrin which play an important immunoregulative role. The basis of the immunomodulating effect of "Gepon" is the activation of monocytes and neutrophils, the induction of interferons and cytokins, strengthening production IgG- antibodies, specific with respect to the causative agents of different infections both of viral and bacterial aetiology. Drug has antipyretic effect and induces regenerative processes. The clinical efficacy and immunomodulating effect of the drug has already been studied with the infectious diseases of viral aetiology [10-12]. The basic purpose of our investigation was the study of its clinical and sanitizing effectiveness of Gepon in children with the AEI mainly of bacterial aetiology. An additional task of the investigation was the study of intestinal microbiocenosis to explain the influence of Gepon on the quantative and qualitative composition of microflora in the dynamics of disease.(disbiosis)

Materials and Design of the Study

The studies were conducted in the clinic for juvenile infections of Russian State Medical University (Moscow), based in the Intestinal Department of G.N.Speranskii Child State Clinical Hospital №9. There were 50 patients under our observation with the moderately severe forms of the AEI in the age range of 13-14 years. All patients entered hospital during the first 2-3 days from the beginning of disease and obtained conventional basic therapy (diet, oral rehydration, enzymes, anti-symptomatic compounds, etc.), 20 of them obtained furazolidone (Control Group) and 30 - furazolidone and "Gepon" (Treatment Group). These patients where not assigned other preparations with etiological effects (e.g. no probiotics, enteros-sorbent, etc.). The Gepon dosing regime was as follows: the drug was given orally as an 0.1% aqueous solution of 1 mg Gepon, given twice a day (daily dose - 2 mg), a 5-7 day course of treatment. The groups of patients were compared with respect to the age, the severities of disease, aetiology, the type of diarrhoea and intestinal complications (enteritis, colitis, etc.). Both groups of patients where predominantly children of

school age (58%) with "invasive" type diarrhoea (76%), enterocolitis in 44% and colitis in 22% of them. The etiological diagnosis of the AEI was made on the basis of clinico- epidemiological and laboratory data, in this case the results of not only bacteriological and serological tests were considered, but also the results of a PCR-diagnosis and study by the quantitative method of seeding the faeces of 20 treatment patients and 15- control patients.

Taking into account the results of these studies, the aetiological diagnosis of the AEI was made in 36 of 50 patients (72%), including Zonne and Flexner shigellesis in 17(34%), salmonellesis in 6 (12%), klebsiellesis in 10 (20%) and the AEI entero-bacterial aetiology in 3 (6%) of patients.

Results and their Consideration

Analysing the basic clinical symptoms and comparing the Treatment Group and Control Group of patients showed that the treatment of moderately severe forms of AIE with furazolidone plus Gepon resulted in a significant ($r < 0,05$) shortening of diarrheic syndrome and the duration of infectious toxicosis symptoms. (Table 1).

Table 1. Mean duration of clinical symptoms of AIE in children depending on type of therapy

	Control Group n=20	**Treatment Group n=30**
SYMPTOM	Duration in days	Duration in days
Infectious toxicosis	4.15±0.19	3.57+0.18*
Weakness	2.85±0.21	2.23+0.10*
Depressed appetite	3.55±0.19	2.23+0.10
Fever	2.75±0.25	2.30+0.18*
Vomiting	1.20±0.22	2.10+0.13
Stomach pains	2.30±0.22	2.10+1.25
Diarrhea symptoms	6.35±0.36	4.35+0.25*

* $p<0.05$

Differences in the average duration of vomiting, stomach pain and the loss of appetite were not statistically significant ($r>0,05$). It was established that in the Treatment Group of patients who had obtained Gepon, the clinical manifestations of toxicosis (apathy, weakness, fever, etc.) disappeared more rapidly, during the first 2-3 days of treatment. By the third day of treatment in the majority of the Treatment Group (63%), the manifestation of toxicosis completely disappeared, while in the Control Group only in 30% of patients had alleviation from toxicosis. However, by the beginning of the 4th day of treatment, the rate of infectious toxicosis symptoms had regressed in both groups of patients. This established the clinical benefit of using combination therapy from the commencement of disease for AEI in children with compounds with immuno-modulative activity.(Figure 1)

Figure 1. Dynamics of regression of the symptoms of infectious toxicosis

	Control Group n=20	Treatment Group n=30
Day of treatment	**Percent of patients who have recovered from infectious toxicosis**	**Percent of patients who have recovered from infectious toxicosis**
2nd	10	20
3rd	30	63
4th	70	70
5th	90	94

Analysis of the dynamics of stool normalization between the two groups of patients from the commencement of disease, showed that the stool normalized more rapidly with the combined therapy of furazolidone plus Gepon. With furazolidone plus Gepon treatment, the complete normalization of stools was achieved by the third day of treatment in 37% of patients, and on the 4th day - in the majority of cases (63%). On the 5th day of furazolidone plus Gepon treatment of AEI in children, clinical recovery with complete stool normalization began at the end of the 5th day of treatment in 80% of patients, while on the 7th day - almost all patients (93%) had recovered. In contrast with the monotherapy by furazolidone, the stool normalization was observed in 20% of patients on the third day and 30% of patients on the fourth day, at the end of the 5-day of treatment more than in half of cases (55%) remained with unstable stool with the pathologic admixtures (mucus, verdure, etc.). Figure 2

Figure 2. Dynamics of normalisation of stools

	Control Group n=20	Treatment Group n=30
Day of treatment	**Percent of patients who have recovered from stool pathology**	**Percent of patients who have recovered from stool pathology**
3rd	20	37
5th	45	80
7th	75	93

On this basis, the treatment of the moderately severe forms of AEI with normal etiopathogenetic monotherapy (standard 5 day course of furazolidone) is effective only in 45% of cases. The addition of immunomodulator Gepon in the combination therapy enhanced the clinical effectiveness of the 5 day course furazolidone to 80% of cases, while with the 7 days course it was enhanced to 93% of cases.

The clinical effectiveness and duration of the acute period of moderately severe AEI during the first 2-3 days of the treatment was also assessed: in the Control Group the positive dynamics of the regression of clinical symptoms occurred in only 60% of cases, while with the addition of the immunomodulator "Gepon" in the combination therapy resulted in the positive dynamics observed in 90% of the patients. (Table 2)

Table 2 Clinical and sanitizing efficacy of treatment of the moderately severe form of AIE in children

	Number (percent) of patients				
	Improvement	**Clinical recovery by duration of treatment in days**			**Elimination of disease**
	Day 2-3	**Day 3**	**Day 5**	**Day 7**	**of AIE**
Treatment Group n=30	27 (90%)	12 (40%)	24 (80%)	28 (93%)	71.4%
Control Group n=20	12 (60%)	4 (20%)	9 (45%)	15 (75%)	46.6%

Moreover, already during the first 2-3 days of treatment with Gepon, the clinical manifestations of toxicosis (apathy, adynamia, decreased appetite, etc.) decreased or completely disappeared in 40% of cases and the clinical effect of Gepon was estimated by us as "outstanding". With monotherapy, improvement was only seen in 20% of the patients. If the clinical benefit was weakly expressed and there was absence of clinical recovery by the end of 5-7 day course of therapy, the treatment was considered ineffective. With the use of combination therapy with Gepon only in 7% of patients was the clinical effect weakly expressed, while with the monotherapy with furazolidone alone, more than half of the cases (55%) were treatment failures.

During the study of the quantitative and qualitative composition of intestinal microflora (disbiosis) as disease progressed, it was established that the majority of patients (80%) in both groups had disbiotic changes in the microflora of intestine which had occurred already during the initial period of illness (2^{nd} to 3^{rd} the day of disease). In the majority of patients, the concentrations of bifidobacteria (80% of cases), of lactobakteria (65% of cases) and of enterococcus (60% of cases) in the feces tested were significantly reduced. The quantity of Escherichiae coli exceeded their normal content in 60% of cases, including lactoso-negative forms in 30% and hemolytic forms in 20%. In both groups it was noted that the concentration of Escherichae and Enterococcus exceeded standard levels. In more than in half of the cases, representatives of conditionally pathogenic causative agents (Klebsiella, Stafilococcus Aureus, and Proteus, fungi Candida) were found in feces test. In the Control Group the number of patients with normal exogenous gut microfloral decreased significantly during treatment: those with normal bifidobacteria decreased from 47% to 13%, and those with normal lactobacteria decreased from 60% to 40%. (Figure 3)

Figure 3. Percent of patients with normal exongenous gut microflora in treatment of AEI in children with furazolidone (Control Group)

	Before treatment	Day 5-7 of treatment
bifidobacteria	47%	13%
Lactobacteria	60%	40%
Escherichia	20%	7%
Enterococci	47%	13%

In contrast by day 5 to day 7 of treatment with Gepon plus furazolidone, the percent of patients with the normal concentration indicators of bifidobacteria (10^8-10^{10} KOE/gram feces) had increased from 20% to 55%, and the percent of patients with normal concentration indicators of lactobacteria (10^6-10^7 KOE/gram feces) had increased from 35% to 80% with Gepon, (Figure 4).

Figure 4. Percent of patients with normal exongenous gut microflora in treatment of AEI in children with fuorazolidonom plus Gepon (Treatment Group)

	Before treatment	Day 5-7 of treatment
bifidobacteria	20%	55%
Lactobacteria	35%	80%
Escherichia	5%	10%
Enterococci	10%	25%

In patients who received combination therapy including Gepon, the microflora feces test showed that patients suffering from depressed levels of bifidobacteria (in 65% of cases) and lactobacteria (in 60% of cases) responded with an increase of 10^2-10^3 or more in bacterial concentration. Patients who had normal initial quantities of microflora (35% of patients had normal bifidobacteria and 30% of patients had normal lactobacteria) Gepon treatment did not result in any negative dynamics. The normalizing influence of Gepon on the quantitative composition of Escherichiae and enterococci was less significant; however a distinct tendency was noted toward the decrease of the elevated levels including an almost complete disappearance of the lactosonegative forms, hemolytic forms of Escherichiae and Enterococci.

Assessment of the treatment schemes for sanitizing effectiveness with respect to pathogenic (Shigella, Salmonella) and conditionally pathogenic microflora (Klebsiella pneumoniae, enterobacter and others.) showed that the sanitizing effectiveness with monotherapy by furazolidone did not exceed 46,6% of cases, while the addition of Gepon in the combination therapy increased the sanitizing efficacy with respect to the growth of these pathogens to 71,4% of cases. A marked sanitizing efficacy with the combination therapy furazolidone plus Gepon occurred with respect to Zonne and Flexner Shigellae in 80% of patients and Klebsiella pneumoniae in

83,3% of patients, to smaller degree - Salmonella in 67% of patients and enterobacteria, in 50% of patients. With furazolidone monotherapy, over-growth of Zonne and Flexner Shigellae was found in 42,8% of patients, over-growth of Salmonella in 66,6% of patients and over-growth of Klebsiella pneumoniae in 75% of patients, and also the growth of enterobacter to high titres (10^6-10^8 KOE/gram) occurred at the beginning of the disease. In the Control Group, the overgrowth of Candida fungi was only reduced in only in 2 of 10 children. In the Treatment Group treated with Gepon, in 5 of 17 patients the overgrowth of the Candida fungi was considerably decreased (by 10^2-10^3 and more) and complete sanitation of intestine from the Candida fungi was found in 4 patients. 53% (9 of 17) of the patients suffering from Candida overgrowth significantly benefited from Gepon .

Therefor the inclusion of the immunomodulator Gepon in combination therapy of the moderately severe forms of AEI in the children increases not only the clinical and sanitizing effectiveness with respect to pathogens, but also contributes to the normalization of the quantitative and qualitative composition of intestine microflora (disbiosis).

Conclusions and the proposal

The addition of the immunomodulator "Gepon" (5-7 day course, a daily oral dose of 2 mg as a 0,1% aqueous solution) plus antibacterial drugs (furazolidone) as a combination therapy for treating moderately severe forms of AEI in children contributes to the following:

- Shortening the duration of the symptoms of infectious toxicosis and diarrhoeic syndrome - the average duration of the acute period of disease reduced from 6,35 $\pm$ 0,36 days (monotherapy with furazolidone alone) to 4,35 $\pm$ 0,25 days (combination treatment with Gepon);
- Increase in the sanitising efficacy of antibacterial therapy (furazolidone) both with respect to pathogenic bacteria (Shigellae, Salmonella) and conditionally pathogenic causative agents of AEI (Klebsiella pneumoniae, enterobakters) from 46,6% of cases to 71,4%;
- The normalisation of the quantitative and qualitative composition of intestinal microflora, mainly bifidobacteri- and lactobacteri, enterococci and the fungus Candida, in contrast to monotherapy by furazolidone which causes progression of disbiotic changes in the microflora of the intestine.

The new immunomodulator Gepon is recommended for clinical practice for the purpose of enhancing traditional eteropathogenetic therapy both for moderately severe and severe forms of AEI in children. Gepon should be used in combination with the standard treatment of broad spectrum antibiotics whose side effects include immunosupression and substantial disruption intestinal microbiological balance in these patients.

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