APPLICATION OF INDUCER OF ENDOGENOUS INTERFERON IN TREATMENT PROTOCOLS OF CHILDREN WITH CHRONIC HEPATITIS B

Kriuchko T.O., Nesina I.M.
Ukrainian Medical Dental Academy, Poltava

Resume. *The objective of this study was to research the efficiency of complex treatment of chronic hepatitis B (CHVB) in children using an antiviral drug with interferon inducing properties – "Proteflazid". Analysis of Proteflazid efficacy in the combined therapy of chronic hepatitis B has shown that in children who used inducer of endogenous interferon, biochemical remission of the virus has been reported sooner, the concentration of IFN of both classes increased significantly, the treatment also contributed to the cessation of replication of the virus. The results of the study showed that in children with chronic hepatitis B changes in the concentration of IFN in the serum were reported that require individual treatment order based on baseline indicators: in patients with low content the drugs with concentrated interferon are recommended, with a high content of IFN-α the treatment should begin with inducers of endogenous interferon.*

Key words: *children, chronic hepatitis B (CHVB), Proteflazid, inducer of endogenous interferon, interferon-α, interferon-γ.*

Introduction

The issue of effective treatment of chronic viral hepatitis in children remains the most complex and not completely solved issue. Recently, the treatment of chronic hepatitis B (CHVB) was limited to backbone therapy, but with the development of medical science a significant number of drugs had emerged that affect not only the immune-metabolic and energy processes in liver cells, but also the virus itself, i.e. the drugs with antiviral action. The main purpose of the treatment of chronic hepatitis B in children is viral eradication and suppression of inflammation caused by it, preventing the progression of the disease and development of liver cirrhosis and its complications [5].

Causal therapy is used in the presence of viral replication and immune responses induced by it [3,10.13]. Antiviral therapy is a promising trend, it is widely used in the treatment of viral hepatitis. Today, concentrated interferons (Intron A, Roferon A, realdyron, viferon, velferon), synthetic nucleosides and interferon inducers [1.6,12] are used primarily as antiviral drugs.

An alternative approach in the treatment of CHVB is using drugs inducers of endogenous IFN. Down to recent times only the use of cycloferon is studied in pediatrics well enough of the inducers of IFN. Along with synthetic inducers of endogenous interferon in applied medicine the drugs began to be used that contain biologically active substances of plant origin with a relatively low toxicity and selectively-specific pharmacological action. These include flavonoids, or flavone vitamins which are a group of biologically active phenolic compounds, the bases of molecule of which is flavonoid heterocycle. It has been definitely found that all compounds of this type have antioxidant properties, but their function is not limited to participation in redox processes [7]. Representative of this group of drugs is "proteflazid" that contains composition of flavonoid glycosides obtained from wild grasses Deschampsia caespitosa L. and Calamagrostis epigeios L. The mechanism of antiviral action of the drug is based on the phenomenon of inhibition of DNA polymerase activity in virus-induced cells as well as induction of endogenous interferon (alpha-, gamma-). Proteflazid particularly stimulates protective processes of the macrophage system, which is an important element in the processes in non-specific defense system. The drug has almost no effect on the not infected cells where there is no increased activity of DNA polymerase. So Proteflazid is blocking the enzyme only in virus-modified cells [7].

The objective of our study was to research efficiency of combined therapy of chronic hepatitis B in children by means of an antiviral drug with interferon inducing properties.

Study material and methods

We examined 47 children aged 8 to 15 years with chronic hepatitis B. The control group consisted of thirty healthy children of similar age and gender. Verification of clinical diagnosis of hepatitis was carried out according to the classification adopted at the World Congress of Gastroenterology (Los Angeles, 1994), taking into account the findings of virological, clinical-laboratory and instrumental studies. To study the therapeutic efficacy of "Proteflazid" as a part of combined therapy, the children with CHVB were divided into two groups. The first group (n=27) were the subjects with chronic hepatitis B who in the process of the combined therapy received antiviral drug "Proteflazid" according to the following dosage regimen: children aged 8-11 - 4 drops 3 times a day for a week; from the 7th day - 8 drops 3 times a day for 3 months, 4-6 months - 10 drops 2 times a day; children aged 12-16 - 5 drops 3 times a day for a week; from the 7th day - 10 drops 3 times a day for 3 months, 4-6 months - 10 drops 2 times a day. The children of the second group (n=20) received conventional pathogenetic therapy.

To evaluate the efficacy of the therapy the criteria recommended by the European Group for the Study of Liver Diseases (Eurohep, 1996) were used:

• initial remission - no replication markers in serum and normalization of ALT levels during the treatment, which is confirmed by two successive tests at intervals of a month, regardless of whether the remission kept up to the end of the treatment;

• stable remission - no replication markers in serum and normal ALT levels during the first 6 months of treatment;

• lack of remission - cases in which after 3 months of treatment positive trend of ALT is absent due to the presence of replication markers;

• recurrence - presence of replication markers in serum and repeated ALT increase in the next 6 months after therapy.

In children of the main group, markers of viral hepatitis (Hbs Ag, Hbe Ag, anti Hbcor IgM, IgG, anti-HbsAg, anti-HbeAg, DNA of HVB) were detected in serum by enzyme immunoassay (IFA) and polymerase chain reaction (PCR) [5]. Changes of interferon profile were evaluated based on the level of serum interferon-alpha (IFN-α) and interferon-gamma (IFN-γ) defined by ELISA ("Vector-Best", Novosybirsk and "Proteinoviy Kontur", St. Petersburg, Russia). The findings were compared in groups before and after treatment, and on a case by case basis in groups of children who received inducer of endogenous interferon - proteflazid.

Processing of numerical data was generally carried out using mathematical methods commonly used in medical statistics. Mean values (M), mean errors (m) of the studied parameters were calculated. Statistical significance was calculated using Student's criterion. The difference of the values were believed to be likely at a value of $p<0.05$.

Study results and their discussion

The leading goal of therapy of CHVB in children is receiving sustained virological and biochemical response [8]. The result of treatment is usually clinical and laboratory remission of the disease and reduction of the progression of fibrotic changes in the liver.

Table 1.

Efficacy evaluation of therapy in children with CHVB (M±m)

Group of children	Remission				Recurrence		Lack of replication	
	stable		absence					
	abs.	%	abs.	%	abs.	%	abs.	%
1 group	12	44.4±9.6 *	9	33.3±9.1 *	4	14.8±6.8	2	7.4±5.0
2 group	3	15.0±7.89	17	85.0±8.9	2	10.0±6.7	0	0

Note: *The difference is significant in comparison with the findings in the children of the other group.

Evaluating the results of our study (Table 1) as recommended by the European Association for the study of liver diseases (EASL 1999) we can indicate that a stable remission was diagnosed in the subjects who used proteflazid as a part of combined therapy, almost three times more often than in children of the second group ($p<0.05$). Lack of remission was reported in 85% of the subjects who received only backbone therapy and only in 33.3% of the children of the first group ($p<0.05$). Inclusion to combined therapy using proteflazid enhances the efficacy of treatment, achieving biochemical and virological remission and is almost three times higher than the results of conventional treatment. Different results of therapy in the subjects with chronic hepatitis B provided treatment using antiviral drug with interferon inducing properties and those who were provided only conventional treatment, due to the peculiarities of their mechanisms of action.

Table 2.

Changes of biochemical parameters in children with chronic hepatitis B during treatment

Biochemical parameters	Children with chronic hepatitis B			
	Before treatment		After treatment	
	1 group (n=27)		1 group (n=27)	
Bilirubin total, μmol / l	36.5±3.7	26.95±1.86	24.0±1.61*	20.55±0.9*
Conjugated bilirubin, μmol / l	14.33±1.87	10.1±1.32	8.1±0.88*	6.3±0.48*
Unconjugated bilirubin, μmol / l	22.13±2.11	16.9±0.8	15.9±0.89*	14.2±0.63
Activity of ALT, μmol / l h	0.83±0.09	0.61 ±0.03	0.6±0.04*	0.5±0.02
Activity of AST, μmol / l h	0.5±0.06	0.4±0.02	0.37±0.03*	0.4±0.02
Diastase	23.0±0.76	20.3±0.63	20.03±0.6	10.9±0.7
Total protein, g / l	69.36±0.96	68.9±1.35	71.37±0.73	69.4+0.05
Albumin, g / l	32.78±0.93	32.4±0.64	54.46±0.63	53.1± 0.59
α 1-globulins, %	4.5±0.25	4.1±1.17	4.53±0.24	4.4±0.23
α 2-globulins, %	9.6±0.4	9.2±0.4	9.2±0.31	9.1±0.5
β-globulins, %	12.7±0.33	12.1±0.7	12.4±0.34	11.95±0.5*
γ-globulins, %	20.2±0.88	20.9±0.39	20.0±0.5	20.5±0.48
Blood test for Hepatitis	1.19±0.03	1.13±0.03	1.2±0.03	1.16±0.03
De Ritis ratio (AST / ALT)	0.62±0.03	0.62±0.04	0.7±0.03	0.74±0.04
Thymol test, unit	3.54±0.41	3.23±0.25	3.39±0.25	3.63±0.14
Cholesterol	4.29±0.1	4.18±0.22	4.2±0.13	3.8±0.1
β-lipoproteins	44.77±1.17	44.92±1.60	44.9±1.39	40.43±1.34*
Prothrombin index, %	86.2±2.16	81.4±1.29	83.59±1.95	86.1±1.32
Recalcification time, sec	104.0±2.87	107.0±3.84	102.96±2.97	105.8±2.33
Fibrinogen, g / l	2.1±0.13	2.18±0.13	2.23±0.11	2.29±0.14

Note: *The difference if significant in comparison with the findings of the subjects before and after treatment ($p<0.05$).

Results of changes of biochemical parameters we used to monitor the efficacy of treatment (Table 2). Under the influence of treatment using proteflazid improvement in functional status of the liver was detected, as evidenced by regress of cytolytic syndrome symptoms: decrease of total bilirubin and its fractions compared with those before treatment (36.5±3.7 μmol / l versus 24.0±1.61 μmol / l, $p<0.05$; 14.35±1.87 μmol / l vs. 8.1±0.88 μmol / l, $p<0.05$; 22.15±2.11 μmol / l vs. 15,9±0.89 μmol / l, $p<0.05$); normalization of transaminases (ALT 0.83±0.09 μmol / l / h vs. 0.6±0.04 μmol / l / h; $p<0.05$; AST 0.5±0.06 μmol / l / h vs. 0.37±0.03 μmol / l h, $p<0.05$). Biochemical parameters also underwent changes during backbone therapy, but in contrast to rates in the subjects of the first group, we have not detected any impact on activity of indicator enzymes. Normalization related more to pigment metabolism parameters (total bilirubin, direct bilirubin).

Thus, analyzing the changes in biochemical parameters during combined therapy using proteflazid, we can say that this therapy helps to restore liver function, increases the secretion of bile, normalizes lipid, carbohydrate and protein metabolism.

Using correlation analysis of biochemical parameters of the subjects in the first group before and after treatment, we found dependence of indicators of cytolytic syndrome and coagulation hemostasis (total bilirubin r=0.58, p<0.001; direct bilirubin r=0.42, p<0.009; indirect bilirubin r=0.39, p<0.05; ALT r=0.44, p<0.02; recalcification time r=0.54, p<0.005; prothrombin index r=0.52, p<0.01). In the subjects of the second group correlation analysis revealed dependence of indicators of pigment metabolism and levels of total protein (serum total bilirubin r=0.46, p<0.05; level of direct bilirubin r=0.55, p<0.01; serum total protein r=0.44, p<0.05).

Comparing the data of the treatment, we can conclude that drugs of pathogenic therapy have almost no influence on the activity of inflammation and bleeding disorders specific to the course of CHVB in childhood. The positive clinical and laboratory dynamics allows to recommend using proteflazid for treatment of children with CHVB to reduce inflammation in the liver, which in turn affects the development of fibrotic changes in the hepatocytes.

According to the data presented in Table 3, a significant increase in content of INF of both classes was reported in the subjects with CHVB before treatment, while the concentration of INF- α was almost 5-fold higher than in the control group. INF-α relates to antiviral cytokines, it stimulates phagocytosis as well, increases the activity of NK-cells and other effector cells of the immune system, induces the production of cytokines, increases the expression of products of main histocompatibility complex [2,4,9].

Table 3.

INF concentration in the examined children (M±m)

Parameters	Children with CHVB			Control group (n=30)
	Before treatment (n=47)	After treatment		
		I group (n=27)	II group	
INF- α, pg/ml	18.50±1.62*	61.96±5.42* γ	31.76±2.22* γ	3.8±0.84
INF- γ, pg/ml	176.54±4.89*	257.46±15.6* γ	197.24±6.86* γ	154.7±3.49

Notes: *difference compared with parameters of healthy children, γ difference is significant in comparison with parameters before treatment.

The nature of the immune response to viral infection depends on the dominant influence of the clones of T-lymphocytes helpers of subclasses 1 and 2, which differ in their range of produced cytokines [9,11,12]. Increased levels of circulating IFN - , which is a part of a set of immunoregulatory molecules characteristic of Th1 clone of T-lymphocytes, can mean enhancing T-helper cells of the first type. This means that Th1-cytokines are actively involved in the pathogenesis of chronic hepatitis [9,13]. In children of both groups positive dynamics of these cytokines was reported during treatment but increased concentrations of IFN in the first group greatly exceeded those of children of the second group. One of the mechanisms of control of the liver fibrogenesis processes is characteristic of production of anti-fibrogenic cytokines by macrophages, these include also IFN-α [13]. IFN-α a is a cytokine of the first phase of the antiviral immune response, it induces inflammation, increases the cytotoxicity of macrophages, T-lymphocytes and natural killer cells that are involved in the mechanisms of natural protection against viral infections [8,11].

No side effects in the treatment of the subjects in both groups were reported.

Conclusions

Despite the fairly large number of publications on interferon therapy of CHVB, there are practically no studies in which the state of IFN system in dynamics in children with CHVB would be analyzed. Results of the study indicate that such subjects have changes in the concentration of serum interferon that require individual treatment order based on initial values of IFN: in the subjects with low content the drugs with concentrated IFN are indicated, with high content of IFN - α treatment should start with drugs with interferon inducing properties. Analysis of laboratory findings showed that combined therapy with the inclusion of Proteflazid was superior to backbone therapy in a number of factors: establishment of clinical and biochemical remission, a significant

increase in the concentration of IFN in the serum and positively influenced termination of virus replication.

Quite a promising direction in the treatment of chronic hepatitis B in children is a combination of drugs with concentrated interferons and interferonogens. In our opinion, further studies aimed at scientific-based regimens in childhood chronic hepatitis B will enable to control the efficacy and forecast the outcomes of the treatment.

REFERENCES

1.Belousov Y.V., Moiseyev V.S., Lepakhyn V.K. Clinical pharmacology and pharmacotherapy. - M.: Universum Publishing, 1997.
2. Dikanskaya N.V. Features of interferon and interferon status of the patients with acute viral hepatitis B and C: Dis. of Cand. Med. Sciences. - M., 1998.
3. Ershov F.I. Interferons // Problems of Virology. - 1998. - issue 6. - P.247-252.
4. Ershov F.I. Interferon system in health and disease. - M., 1996.
5. Diagnostic criteria and principles of treatment of chronic hepatitis B in children: Method. recom. /Lukyanov O.M., Belousov Y.V., Denisova M.F. et al. -. K., 2003.
6. Malinovskaya VV, Neumoina MV, Sorinson S.N. // New application prospects of interferon drugs in pediatrics and gynecology: Coll. scientific-practical works. Conf. - St. Petersburg, 1997. - P.31
7. Proteflazid: Scientific-practical recomm. - K., 2003. - 89 p.
8. Reyzys A.R., Nikitina T.S., Drondina A.K. Study of Viral Hepatitis in the clinical department of children // Epidemiologia i infectsionnye bolezni, - 1999. – issue 2. -P.46-48.
9. Th1-cytokines during chronic viral forms hepatytov B and C / Pryymyahy L.S., Tefanova V.T., Tallo T.G. et al. // Problems of virology. - 2003. –issue 3. -P.23-27.
10. Uchaikin V.F., Kovalev O.B. Treatment of acute and chronic hepatytis in children acc. to program of a protocol // Russian journal of gastrozhterol., hepatol., koloproktol. - 2001. - Spec. Issue. - P.54-57.
11. Levin S., Hahn T. // Clin.Exp. Immunol/ - 1995. - Vol. 60. - P. 267-273.
12. Pirovino M., Aguet M., Huber M. // Hepatology. - 1996 - Vol.60. - P.767- 771.
13. Quin J.W. //Aust. N.Z.J. Med - 1997 - Vol.27. - P.611-618.