UDC 577.121:616.36-002-053.2

Metabolism features in the liver cells in children depending on the stage of chronic hepatitis B

Bulygin V.G.[1], Bulygin G.V.[2]

The study included 24 children between the ages of 12 and 16 years of age, patients with chronic hepatitis B (14 men from the 2 stage of the chronic process, and 10 - with the third stage), in which a liver biopsy determined for the activity of NAD/F/-dependent enzymes and parameters of lipid profile. We found that the increase in chronic stage of infection was accompanied by a decrease in liver cells of cholesterol and phospholipids, lipid shift toward the formation of triacylglycerides, decreased activity of NADPH-dependent enzyme and decrease the synthetic potential of cell metabolism and their proliferative capacity, a reduction in the protection of cells from peroxidation processes lipid reduction in their ATP production by inhibiting the reactions of the Krebs cycle and glycolysis.

Keywords: children, chronic viral hepatitis B, chronic stage, the liver, lipids, enzymes, metabolism.

Introduction

Analysis of morbidity and economic costs of chronic viral hepatitis B [9] allows us to consider the disease as one of the priority issues of infectious diseases in Russia [10]. Particularly acute issue for pediatric practice, as it is one of the most important cause of childhood disability [3].

Despite numerous studies conducted in chronic hepatitis B, and the success achieved in the study of the pathogenesis of this disease [1, 7, 8], many issues have not been resolved definitively. Of great interest is to obtain information directly to the metabolic changes in the liver cells of patients with different stages of chronicity of the disease.

Manifestation of features of any cell of a living organism is largely determined by its intracellular metabolism, depending on the specific disease process. Parameters that reflect the direction and intensity of the reactions in the cells, which are defined in the lipid profile and enzyme activity [2]. In the available literature is not enough information about the research in chronic

hepatitis B in children, evaluating the metabolism in the liver of these parameters, however, such information is very important to better understand the features of the pathogenesis of this disease.

The aim of our research - definition of lipid composition and activity of enzymes in the liver in children with chronic hepatitis B and chronic study metabolic mechanisms of this disease.

Materials and methods

24 children at the age of 12-16 years with diagnosis "Chronic virus hepatitis B" which was established in the conditions of a specialized hospital by means of a set standard clinic-biochemical, and also immunofermental methods of research are surveyed and was confirmed morphologically at a puncture biopsy of a liver (under ultrasonography control) taking into account the histological index of degree of activity (HIDA) and the histological index of a stage of synchronization (GISS) – according to V.V. Serov [6]. From all surveyed children at 14 people the 2 stage of synchronization of process (weak or moderate degree of activity decided on moderately expressed fibrous changes in a liver) and at the 10 – the 3 stage of synchronization (weak or moderate degree of activity with heavy fibrosis in a liver).

The bulk of the liver tissue obtained by biopsy was used for histological conclusion, and 3-5 mg of it - for the determination of lipid composition and activity of intracellular enzymes.

Parameters of lipid profile of liver cells - phospholipids (PL), cholesterol (CHOL), free fatty acids (FFA), triacylglycerides (TAG) and cholesterol esters (EH) - determined by thin layer chromatography on silufole with extraction of lipids by J. Folch et al. [11] followed by densitometry to determine the ratio (%) of each of the lipid fractions.

Bioluminescent method [5] determined the activity of metabolic enzymes: glucose-6-phosphatdehydrogenase (G6PDG), glycerol-3-fosfatdegidrogenase (G3PDG), lactatdehydrogenase (LDH), NAD- and NADP-dependent isocitratdehydrogenase (NADIDG and NADFIDG), NAD- and NADP-dependent glutamatdehydrogenase (NADGDG and NADFGDG), NAD- and NADP-dependent malatdehydrogenase (NADMDG and NADFMDG) and glutathionreductase (GR). The enzyme activity was expressed in micrograms per 1 microunits liver tissue (mkE/mg).

These data are processed by methods of statistical analysis using the software package Statistica 6,0 and recommendations for their use in biological and medicine [4]. The table shows the mean group values (M) and the error of the mean (m). The significance of differences was assessed non-parametric Mann-Whitney (U).

Results

The study revealed significant differences between the lipid composition of the liver cells of the patients in groups of children differing in stages of chronic hepatitis B (table 1).

Thus, in the liver in children with stage 3 chronic infection is determined on a lower level than in the 2 stage, the percentage of the main structural components of cells - PL (11,65 ± 1,58 and 16,67 ± 1, 35, p <0.05) and CHOL (12,50 ± 1,30 and 19,24 ± 1,67; p <0.01). At the same time, in the third stage of a higher proportion of TAG: 36,54 ± 3,06 and 27,46 ± 2,55; p <0.05.

Lower concentration in the liver of the main structural lipids (CHOL and PL), the bulk of which is synthesized in this body, in children with the third stage of chronicity is probably a reflection of the rise, compared with the 2 stage, a functional deficiency in the body worsening severity of the process. Given the crucial role of CHOL in limiting lipid peroxidation reactions, reduction of this fraction in the cells, of course, reduces the possibility of their antioxidant defense and creates conditions for even more damage to the liver cells multifactorial pathological process.

However, the most important of the established us in this part of the study metabolic changes can be considered that the study of indicators confirm the shift towards lipid TAG intensive education by limiting the exchange of CHOL and reducing the share of the FFA. This indicates that at the third stage of chronic hepatitis B more active mechanism of lipid damage to the cells of the liver due to accumulation of TAG in them.

When comparing the activity of the enzymes in the liver cells at different stages of chronic hepatitis B revealed differences between the majorities of indicators (table 2). It was found that the third stage of chronic metabolic intensity of intracellular events in the liver is lower than in stage 2: G6PDG (7,99 ± 2,36 and 23,63 ± 6,09, p<0, 05), NADFIDG (19,06 ± 5,30 and 51,88 ± 10,63; p <0.05), NADFGDG (9,48 ± 4,26 and 31,53 ± 8,20; p <0, 05), NADFMDG (7,90 ± 1,84 and 22,23 ± 3,83; p <0.01), GR (3,01 ± 0,81 and 7,29 ± 1,58; p <0, 05), NADMDG (77,75 ± 17,94 and 199,91 ± 42,73; p <0.05) and LDH (7,18 ± 2,26 and 20,73 ± 4,43; p <0, 05).

General fact attracts attention is the fact that in patients with stage 3 chronic all investigated NADPH-dependent enzymes are determined less active. Inhibition of these enzymes in the cells limits the formation of reduced NADP, and therefore less able to form intracellular metabolism to the reductive synthesis, including the synthesis of fatty acids and steroids (which is combined with the characteristics stated above lipid metabolism). Less high than at stage 2 chronic, activity of G6PDG - pentose phosphate pathway enzyme - limits not only the quantity produced in the liver cells of reduced NADPH, but also reduces the possibility of the way to ensure an adequate synthesis of nucleotides and nucleic acids, and in the end - cell proliferation .

In addition, when the third stage below the level of protection of liver cells from lipid

peroxidation: reduced activity of GR - the antioxidant enzyme glutathione system. Given the role of the GR in the transport of amino acids into the cell, it can be assumed that their entry into the cells of the liver in children with stage 3 is much smaller than the 2.

At the third stage of chronic hepatitis B in liver cells is lower than in stage 2, and the level of production of energy substrates. This is evidenced by less high activity of enzymes of the Krebs cycle, which produces the bulk of the cells in the ATP needed to power their life. These include both primary dehydrogenase and the end of the cycle (NADFIDG, NADMDG, NADFMDG) and enzyme NADFGDG serving the loop substrates with amino acid metabolism. Thus, the efficiency of the Krebs cycle to ensure the functional needs of the liver cells in the third stage is reduced.

At the same time in the cells of the liver in patients of this group is not determined and the possible compensatory reaction in the form of increasing the intensity of glycolysis, which is "emergency mechanism», can occur in conditions of increased functional load on the cells for their needs in the ATP. This is supported by lower activity of LDH in the third stage of chronicity, which not only reduces the production of ATP, but also contributes to the accumulation of lactate in the cells and the formation of intracellular acidosis.

Conclusion

As a result, our research found that in children 12 to 16 years of age, patients with chronic viral hepatitis B, metabolic changes in the liver caused by rise of chronic stage of the disease, manifested by reduced major structural lipids - CHOL and PL shift towards lipid lipogenesis and TAG accumulation in cells, decreased activity of NADPH-dependent enzymes and reduced synthetic capacity of intracellular metabolism and proliferative capacity of the liver cells, decrease in their ATP production by inhibiting the reactions of the Krebs cycle and glycolysis, as well as a reduction in the protection of liver cells from peroxidation lipids.

References

1. Bulygin V.G. Tihonova E.P. Aksenova N.A. Bulygin G.V. Parametry metabolizma v limfocitah detej i vzroslyh, bol'nyh hronicheskim virusnym gepatitom B [Metabolic parameters in lymphocytes of children and adults with chronic viral hepatitis B] Sibirskoe medicinskoe obozrenie [Siberian medical review]. 2010. №2. pp.33–36.

2. Bulygin G.V. Kamzalakova N.I. Andrejchikov A.V. Metabolicheskie osnovy reguljacii immunnogo otveta [Metabolisms framework regulation of the immune response]. Novosibirsk: SB RAMS, 1999, 346 p.

3. Gunjakova V.K. Virusnyj gepatit B u detej: kliniko-jepidemiologicheskie i social'nye aspekty [Viral hepatitis B in children: clinical and epidemiological and social aspects] Rossijskij pediatricheskij zhurnal [Russian Journal of Pediatrics]. 2005. №3. pp.21–26.

4. Rebrova O.Ju. Statisticheskij analiz medicinskih dannyh: Primenenie paketa prikladnyh programm Statistica [Statistical analysis of medical data: Application software package Statistica]. Moscow: Mediasphere, 2002. 312 p.

5. Savchenko A.A. Suncova L.N. Vysokochuvstvitel'noe opredelenie aktivnosti degidrogenaz v limfocitah perifericheskoj krovi cheloveka bioljuminescentnym metodom [Highly sensitive determination of dehydrogenase activity in human peripheral blood lymphocytes by bioluminescent] Laboratornoe delo [Laboratory work]. 1989. №11. pp.23–25.

6. Serov V.V. Severgina L.O. Morfologicheskie kriterii ocenki jetiologii, stepeni aktivnosti i stadii processa pri virusnyh hronicheskih gepatitah B i C [Morphological criteria for assessing the etiology, and the degree of activity of the process in viral chronic hepatitis B and C] Arhiv patologii [Archives of Pathology]. 1996. №4. pp.61–64.

7. Solov'eva I.A. Savchenko A.A. Martynova G.P. Sostojanie immunnogo statusa u detej s hronicheskimi virusnymi gepatitami B i C [Of the immune status in children with chronic viral hepatitis B and C] Tihookeanskij medicinskij zhurnal [Pacific Medical Journal]. 2010. №1. pp.55–57.

8. Tihonova E.P. Bulygin G.V. Metabolicheskie osnovy immunoreaktivnosti pri parenteral'nyh gepatitah B i C [Metabolic basis of immunoreactivity with parenteral hepatitis B and C]. Novosibirsk: Nauka, 2003. 148 p.

9. Shahanina I.L. Osipova L.A. Jekonomicheskie poteri ot infekcionnoj zabolevaemosti v Rossii: velichiny i tendencii [Economic costs of infectious disease in Russia: the magnitude and trend] Jepidemiologija i infekcionnye bolezni [Epidemiology and Infectious Diseases]. 2005. №4. pp.14–25.

10. Shahgil'djan I.V. Jasinskij A.A. Mihajlov M.I. i dr. Hronicheskie virusnye gepatity v Rossijskoj Federacii [Chronic viral hepatitis in the Russian Federation] Kliniko-jepidemiologicheskie i jetno-jekologicheskie problemy zabolevanij organov pishhevarenija: materialy vos'moj Vostochno-Sibirskoj gastrojenterologicheskoj konferencii s mezhdunarodnym uchastiem i Krasnojarskoj kraevoj gastrojenterologicheskoj konf., 17–18 aprelja 2008 g. [Clinical and epidemiological and ethno-ecological problems of digestive diseases: Materials eighth East Siberian gastroenterology conference with international participation and the Krasnoyarsk regional gastroenterology conference., 17–18 April 2008 – Krasnoyarsk, 2008]. pp.246–253.

11. Folch J. Lees M. Sloane-Stanley G. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 1957. Vol.226, N1. pp.497–509.

Table 1.

The lipid content (%) in the liver of children at different stages of chronic hepatitis B, (M ± m)

Indicators	Stage 2 chronic, n=14	Stage 3 chronic, n=10
PL	16,67±1,35	11,65±1,58 p<0,05
CHOL	19,24±1,67	12,50±1,30 p<0,01
FFA	9,34±1,37	6,72±0,80
TAG	27,46±2,55	36,54±3,06 p<0,05
EH	28,33±1,89	32,59±0,74 p<0,1
CHOL/PL	1,24±0,05	1,12±0,09
FFA/TAG	0,42±0,09	0,21±0,04 p<0,1

Table 2.

The enzyme activity (mkE/mkg) in the liver of children at different stages of chronic hepatitis B, (M ± m)

Indicators	Stage 2 chronic, n=14	Stage 3 chronic, n=10
G6PDG	26,63±6,09	7,99±2,36 p<0,05
G3FDG	132,78±16,07	121,09±13,60
LDH	20,73±4,43	7,18±2,26 p<0,05
NADIDG	5,25±1,40	2,30±0,78
NADFIDG	51,88±10,63	19,06±5,30 p<0,05
NADGDG	300,30±66,25	153,29±31,39 p<0,1
NADFGDG	31,53±8,20	9,48±4,26 p<0,05
NADMDG	199,91±42,73	77,75±17,94 p<0,05
NADFMDG	22,23±3,83	7,90±1,84 p<0,01
GR	7,29±1,58	3,01±0,81 p<0,05

The authors:

1. Bulygin Vadim Gennadevich, the candidate of medical sciences, the leading researcher of Federal State Budgetary Institution Research Institute of Medical Problems of the North Siberian Office of the Russian Academy of Medical Science.

2. Bulygin Gennady Viktorovich, doctor of medical sciences, professor, professor of chair of clinical immunology of Public Educational Institution of Higher Professional Training Krasnoyarsk State Medical University named after prof. V.F. Vojno-Yasenetsky of the Ministry of Health and Social Development of the Russian Federation.

The author for correspondence and the correspondence direction:

Bulygin Vadim Gennadevich, the candidate of medical sciences, the leading researcher of Federal State Budgetary Institution Research Institute of Medical Problems of the North Siberian Office of the Russian Academy of Medical Science.

Working address:

660022, Krasnoyarsk, Partizana Zheleznyaka St., 3-g.

E-mail office : impn@impn.ru, ph. office. : (391)228-06-83.

Home address:

660022, Krasnoyarsk, Aerovokzalnaya St., house 2-k, quarter 36.

Ph. house. : (391)276-58-72

E-mail house. : bulyginvadim@mail.ru.