UDC 616.36-002.2-002.7:578.891

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Proteflazid: the specific activity against the hepatitis C virus in preclinical studies; efficacy and safety in the treatment of hepatitis B and C in clinical practice (systematic review)

Abstract

A systematic review of the literature shows the specific activity of Proteflazid against the virus of hepatitis C in a pre-clinical study. The safety and effectiveness of Proteflazid® (drops) and Flavozid® (syrup), in clinical practice in the treatment of hepatitis B and C among children (175) and adults (846 patients, including 262 pregnancy cases) has been confirmed. The results of independent research correspond and demonstrate the effectiveness and safety of Proteflazid.

Keywords: Proteflazid®, Flavozid®, hepatitis B and C, efficiency, safety, comparability of treatment results.

INTRODUCTION

Chronic hepatitis B and C (HBV, HCV) is one of the most urgent problems of modern medical science and practice due to their wide distribution, severe consequences of the disease, such as cirrhosis and hepatocellular carcinoma, decreased quality of life and, in a significant percentage of cases, patients disability.

According to the Medical Statistics Centre of the Ministry of Health of Ukraine, the incidence of chronic viral hepatitis in the past 10 years has grown by an average of 70%, hepatic cirrhosis – by almost 25%, which reflects not only the medical but also social significance of the problem.

At the present stage of development of medical science the protocols for treatment of chronic viral hepatitis include two main areas, the first of which involves the use of drugs to ensure the activation of mechanisms that block the replication of the virus, and the second involves an impact on the pathogenic mechanisms of the disease and its consequences.

Modern standards of chronic hepatitis C treatment include a combination of pegylated interferon-α (PegIFN-α) and ribavirin (RBV). In 2011 telaprevir and boceprevir were approved for use in the treatment of infections caused by the genotype 1 hepatitis C virus (HCV). These drugs are 1st generation antiviral direct action agents; they belong to a group of protease inhibitors and prescribed in combination with PegIFN and RBV. All patients before antiviral therapy require a quantitative determination of HCV RNA using a sensitive molecular genetic methods (lower detection limit <15 IU/mL).

In accordance with clinical guidelines of the European Association for the Study of Liver Diseases the specific therapy should be promptly assigned to patients with severe liver fibrosis and in patients with symptomatic extra-

hepatic manifestations. For patients with no fibrosis or minimal fibrosis the starting date of a specific therapy is controversial and may be postponed until the development of new treatment methods [1].

Antiviral therapy involves the prescription of antiviral substances of direct or mediated (indirect) action. The specific direct action antiviral activity is the ability of a drug to inhibit the reproduction of certain viruses. The drug may have the mediated antiviral effect increasing the activity of the immune system. This particular review considers the hepatitis B virus (DNA-containing Orthohepadnavirus Hepadnaviride family virus) and hepatitis C virus (RNA virus genus Flaviridae family).

The search is ongoing for new therapeutic strategies aimed at improving the efficiency, pangenotypic activity, reducing the duration of treatment, simplification of drug administration mode, improving tolerability, and patients' compliance with treatment.

The antiviral drugs correspond to high therapeutic requirements, the active ingredients of which include flavonoids of Proteflazid extract: Proteflazid® (drops) and Flavozid® (syrup) of «SMC "Ecopharm", Ltd., Ukraine [2, 3].

The extract is obtained by alcohol extraction technology (96% ethanol) from plants (wild grasses - Calamagrostis epigeios L. and Deschampsia caespitosa L.). The drug contains flavonoids extracted from cereals (flavonoid glycosides and flavonolovye), presented in the form of stable molecular complexes – tricine, apigenin, luteolin and quercetin [3].

STUDY OBJECTIVE

To demonstrate on the basis of the available scientific data the specific activity of Proteflazid against HCV in a pre-clinical study. To analyze the independent clinical studies for effectiveness and safety of Proteflazid® (drops) and Flavozid® (syrup) in a clinical setting for the treatment of hepatitis B and C.

MATERIALS AND METHODS

Scientific publications on the preclinical and clinical studies; systematic analysis.

RESULTS AND DISCUSSION

S. L. Rybalko (2004) in preclinical in vitro studies using experimental model of HCV-infection on Rat Gasser's Ganglion Neurinoma Cell Culture (RGGN) studied the activity of Proteflazid ® against HCV. Using the PCR method the following results were obtained: maximum tolerable concentration of the drug to the cell culture - 19.5 pg/mL; minimum concentration of the drug active against HCV – 0.01 g/ml, chemotherapeutic index (CTI) of the drug - 1950. These indicators allow researchers to classify Proteflazid as highly active antiviral agent of direct action against the hepatitis C virus [4, 5].

Further Proteflazid® antiviral activity of the drug was confirmed with viral-cell model of surrogate HCV-infection using a bovine viral diarrhea virus (BVDV), which, like HCV, refers to Flaviviridae family. Studies were carried out on cell line MT-4 (lymphoblastoid cells). The undiluted blood plasma of hepatitis C patients having HCV RNA in various concentrations was used as

the material containing HCV. During the studies the producing culture of MT-4 cells were obtained transfected with complementary DNA (cDNA) HCV and enabling stable production of hepatitis C virus. It has been shown that the drug in a concentration of 0.37 μg/ml inhibited HCV reproduction. The maximum tolerable concentration of the drug was 825 μg/ml, minimum active concentration - 0.37 μg/ml, and the chemotherapeutic index was 2.230 [4, 5].

Similar results were obtained in studies by D.B. Starosila (2014). To investigate the relative activity of Proteflazid relative to HCV the surrogate virus was used of hepatitis C – BVDV and DNA transfected HCV culture of MT-4. It is shown that Proteflazid® drug concentration of 0.375 μg/ml effectively inhibits the expression of HCV RNA and surrogate BVDV reproduction [3].

Yu. I. Porva (2010) studied the activity of Proteflazid® on mixed infections model. For this purpose, the Cell Culture RGGN pretreated with different doses of Proteflazid® was infected with HIV, and then HCV. Viral load was determined by PCR. It was noted that in mixed infections of HIV + HCV HCV viral load increases significantly (from 2450 to 4062 g/eq). It was found that Proteflazid in doses of 2 μg/ml, 1 μg/ml, 0.2 μg/ml significantly (100%, 88%, 100%) inhibits HCV reproduction in mono- and mixed HCV + HIV infection model [5].

It should be noted that the spectrum of the direct antiviral effect of Proteflazid® extends to other viral infections, including those caused by the herpes virus of the 1st and 2nd type, influenza virus, Epstein - Barr virus, HIV, cytomegalovirus, papillomavirus (1, 16 , 31, 35, 39, 59 types) and adenovirus (serotype 5) [3, 6, 7].

According to L. G. Palchikova et al. (2013) flavonoids are target for viral enzymes – transcriptase and protease. The high inhibitory activity was established f the active ingredient of Proteflazid® on in vitro viral RNA synthesis in model transcription system of bacteriophage T7 (T7 RNAP). Proteflazid® is an active inhibitor of T7 RNAP transcription complex with the index IC_{50} (half maximal inhibition concentration) of 0.07 μg/ml. Also the data was presented on Proteflazid® extract effect on the DNA fragments synthesis in PCR conditions (as a model system for screening and selecting agents interrupting or blocking DNA synthesis). Similarly to the RNA synthesis inhibition the concentration-dependent inhibition is observed of the amplification process: IC_{90} value at which the active substance completely inhibits DNA synthesis is 8 μg/ml. The molecular docking was performed to clarify the Proteflazid® blocking sites of DNA and RNA viruses synthesis of fixing process of the extract components to molecular model of RNAP T7 transcription complex. The authors have shown that the compound is held by strong coordination bonds between the oxygen atoms of magnesium ion with an amino acid residue tyrosine 639 of the transcription complex [6].

Proteflazid® demonstrates effective antiviral activity against HCV with a low level of toxicity, as evidenced by the high values of indicators CTI - 680.

It should be noted that the indicator of RNA synthesis inhibition level with Proteflazid® is very close in value to the performance indicators of in vitro viral suppression reproduction [6].

One of the mechanisms of antiviral action of Proteflazid® is interferonogenic activity. According to M. P. Zavelevich et al. (2003) the drug dose-dependently stimulates in vitro the production of interferon (IFN) in human

leukocytes and in continuous culture MDBK cells. Typing indicates that the drug is an inducer of α- and y-IFN [8]. Studies have confirmed that Proteflazid® can induce IFN both in vitro, and in vivo, thus providing an indirect effect on the viruses [2, 3, 7].

In addition to the antiviral activity of Proteflazid® active ingredient the drug exhibits antioxidant properties: increases the resistance of cells to free radical stress in infections, reduces the negative effects of drug chemotherapy, and helps the body to adapt to adverse environmental conditions. It was proved in in vitro experiment that the drug is more than 2-fold inhibits the free radical processes intensity, induced by hydrogen peroxide. At the cellular level, it was shown that Proteflazid® inhibits the generation of superoxide radical anion to almost zero for 24 hours from the time of drug administration, i.e., supports the cell antioxidant status [2].

Proteflazid® also shows the apoptosis modulating activity – contributes to the primary prevention of cancer diseases against the background of chronic (latent) viral infections. Active ingredient of Proteflazid® extract exhibits apoptosis-inducing effect through the activation of initiator caspase 9: restores the ability of cells infected with a virus, to apoptosis, reducing the activity of proliferative processes in the mutated cells [7].

In terms of acute toxicity the herbal antiviral drugs containing flavonoids - Proteflazid® (drops) and Flavozid® (syrup) belong to the 5th class of relatively safe substances. The pre-clinical studies have not revealed teratogenic, mutagenic, embryotoxic, carcinogenic and cytotoxic activities [2, 3, 7].

The agents containing Proteflazid extract are used in clinical practice for the treatment of hepatitis B and C: Proteflazid® (drops) and Flavozid® (syrup). Both dosage forms can be used for adults and children, but to treat the viral hepatitis in children under the age of 12 years it is recommended to use a paediatric formulations – Flavozid® syrup.

Proteflazid® (drops) efficacy and safety in adults.

In the pre-registration stage of Proteflazid® (drops) study in the department of intensive care and detoxification at the L. V. Gromashevsky Research Institute of Epidemiology and Infectious Diseases (Public Institution) of NAMS of Ukraine under the supervision of Doctor of Medical Sciences V. I. Matyash (2000) the controlled (as previously approved by the Protocol of Pharmacological Committee of the Ministry of Health) open clinical study was carried out on the efficacy and tolerability of Proteflazid ® (drops) in 30 patients with hepatitis B and C. The drug was administered according to the scheme: Week 1 - daily 5 drops 3 times a day; Week 2 and 3 – daily, 10 drops 3 times a day; Week 4 – daily, 8 drops 3 times a day for 4 months without interruption. All patients participating in the study were prescribed a diet therapy (diet No. 5). Hepatoprotectors, antispasmodics, regulators of the upper digestive tract motor function, sorbents, diuretic, detoxifying agents and other symptomatic agents were prescribed on indications. No drugs were prescribed in the study with antiviral effect, antimetabolite, immunosuppressive, immunomodulatory agents. It was established that patients receiving Proteflazid® (drops), as compared with a control group of patients, had general health disorders resolved by an average of 7.1 days earlier, and 4.8 days earlier – nausea and dyspeptic disorders. These patients showed a significant decrease in the density of the damaged liver and its

Complex therapeutic effect of Proteflazid® (drops) on biological processes counteracts the chronic process, fibrosis development in the liver [10, 11].

size at the end of treatment (70% of patients taking the drug, versus 59.9% in the control group). The positive result of the liver function recovery is evidenced by the fact that the patients receiving Proteflazid® (drops), by the end of the treatment period had aminotransferases (ALT and AST) decreased by an average of 80%, while in the control group – by 60%, indicating reduced cytolytic components – indicators of chronic process in hepatitis. At the same time Proteflazid® (drops) positive effect was observed in liver protein-synthetic function recovery, as evidenced by a more rapid increase in blood albumin in patients who received this drug [9].

V. I. Matyash et al. (2002), based on experience of using Proteflazid® (drops) in 45 patients with acute and chronic hepatitis B and C, concluded that the drug is etiopato-genetic product for treatment of hepatitis B and C, including their acute forms (basic symptomatic therapy included plasmapheresis, glucose-saline solutions, amino acids). Use of the drug has a positive effect on the recovery of structural and functional state of the affected liver and its homeostatic functions (detoxification, excretion, protein synthesis) causes regression of clinical syndromes (cholestatic, cytolytic, dyspeptic, asthenic).

V. I. Matyash (2003) has subsequently performed a research project on the "Development of new etiopathogenic therapies for viral hepatitis B and C", where, to treat 60 patients with acute and chronic hepatitis B, B + C (20 patients) and B + D (5 patients), Proteflazid® (drops) and its combination was also used with the reference treatment method – discrete plasmapheresis against the background of symptomatic therapy. Based on the result analysis it was established that, subject to continuous administration of the drug for 3 months, the regression of pathological process with liver structure recovery observed in 80% of patients with complicated acute hepatitis B and 70% of patients with chronic hepatitis B treated with Proteflazid® (drops) for 6 months. [12]. Simultaneous infection with hepatitis viruses B and D (HDV) is more severe compared to just one virus infection. Since HDV is HBV satellite, i. e. is capable of reproducing only in HBV-infected cells, it is possible to assume an indirect effect of Proteflazid on HDV by inhibiting HBV reproduction.

Summarizing the experience of Proteflazid® (drops) in the treatment of complications of acute and chronic hepatitis B and B + C, V. I. Matyash et al. (2004) have confirmed that the drug against the background of pathogenetic therapy (glucose-saline solutions, vitamins, Glutargin, cardiovascular drugs, and in the most severe cases – Refortan, plasmapheresis) has a positive effect on the regression of clinical indicators of disease and kinetics of hepatic homeostatic functions recovery (detoxification, excretory, protein and synthetic), and eliminates the cytolytic cholestatic syndromes, providing a more effective reduction in bilirubin and transaminases levels. It is indicated that Proteflazid® (drops) is easily tolerated, does not cause negative side effects both in acute and chronic viral hepatitis, can be successfully used in patients with co-morbidities, including the presence of contraindications to the use of interferon [13].

From a scientific point of view, it is reasonable to use concurrently in the treatment of viral hepatitis both exogenous interferon and Proteflazid® (drops), which in addition to the antiviral action, has the ability to induce the synthesis of endogenous α- and y-IFN. The effectiveness of this approach is

illustrated in the work by A. L. Ivakhiv et al. (2004). The study conducted in the I. Ya. Gorbachevsky's Ternopil Medical Academy was aimed at the efficacy of complex treatment of patients with CHB and CHC with low molecular weight inducer of endogenous IFN cycloferon and its combination with Proteflazid® (drops). It was found that patients with chronic hepatitis C who received Proteflazid® (drops) and cycloferon had biochemical remission earlier as compared with patients who received cycloferon alone. Thus, upon hospital discharge their serum bilirubin was 1.5-fold lower. Patients with chronic hepatitis B had even more pronounced positive dynamics of biochemical parameters: ALT activity decreased to 0.9 mmol/l h vs 1.4 mmol/h-l in the control group. The lengths of patients stay at the hospital reduced (respectively 16.7 and 28.5 per bed-day). The authors emphasize that the combined use of drugs and concentrated exogenous interferons and interferon inducing agents that include Proteflazid® (drops) is a promising way of improving the treatment of chronic hepatitis B and C [14].

In general, similar data regarding the efficacy and safety of Proteflazid® (drops) were obtained by employees of the Kharkiv Medical Academy of Postgraduate Education (P.V. Nartov, O.V. Volobuyev, 2003), who used the drug in addition to conventional basic therapy during treatment of 35 patients with acute hepatitis C. In these patients, above all, it was noted a decrease in the severity of intoxication syndrome, improved appetite, resolved general weakness and nausea. Further case follow-up of these patients showed that 80% of patients had ALT levels returned to normal in 1 month, while in 82% no HBV was found in blood by PCR [15].

In Ivano-Frankivsk Medical Academy Proteflazid® (drops) study was conducted by B.M. Dikiy et al. (2003). In the study 17 patients (study group) received against the background of traditional therapy Proteflazid ®, 20 patients (control group) – traditional therapy. It was found that in a week after prescription of Proteflazid® in patients with prolonged hepatitis B they had manifestations decreased of asthenovegetative, dyspeptic, jaundice syndromes, and in 2 weeks had a significant decrease in the manifestations of cytolytic, mesenchymal-inflammatory syndromes and manifestations of cholestasis. Full normalization of biochemical parameters was observed in 84.4% of patients versus 68.7% in the control group [16].

It should be noted that there is evidence on the effective use of Proteflazid® in the treatment of hepatitis A (HA). So, V.G. Savelyev, V.V. Bondareva (2005) on the basis of Zaporozhye State Medical University conducted a study involving 65 patients with viral hepatitis of various etiologies. Of those, there were 30 patients with HA, and 6 patients with GA against the background of CHC. It was found that treatment with Proteflazid® against the background of basic therapy of acute HA caused in 83.4% of patients a more rapid decrease in symptoms of intoxication, reducing the period of jaundice, improving overall health. 76.8% of patients reported a decrease of cytolytic and cholestatic syndrome. 79.6% of patients showed a positive dynamics of the level of bilirubin, 52% of patients had improvement in bowel function and more rapid disappearance of dyspeptic symptoms. In 18% of patients with GA occurring against the backdrop of CHC and CHC patients after administration of the drug reported the transient enhancement of cytolytic syndrome for 5-7 days, and then the positive dynamics of biochemical parameters and a decrease in liver size (according to US data) [17].

Efficacy and safety of Proteflazid ® (drops) and Flavozid® (syrup) in children

A significant part of the available publications is devoted to the analysis of the results of using Proteflazid® (drops) and Flavozid® (syrup) in the treatment of hepatitis B and C in children. At the Hospital Pediatrics and Pediatric Infectious Diseases Department of State Higher Educational Institution "Ukrainian Medical Dental Academy" (Poltava) the experience was analyzed of treating children with hepatitis B and C using Proteflazid® (drops). Thus, T.A. Kryuchko, I.N. Nesina (2002) in the study of the efficacy of Proteflazid® in treating children with chronic hepatitis B reported the regression of clinical symptoms, normalization of biochemical parameters, reduction of inflammation in the parenchyma, hepatic physiological structure recovery in a shorter time in the group of children treated with the drug compared with the control group of children [18]. Further studies have shown that children who received Proteflazid® (drops) had more rapid biochemical remission, significantly increased α and y-IFN concentration, which is indicative of stimulating own protective mechanisms [19-21].

Mohamed M.A. Abdalaal (2006) demonstrated the effectiveness of the complex therapy with Proteflazid® (drops), membrane stabilizer Thiotriazoline in basic etiopathogenic therapy (enterosgel, gepabene, Galstena diet number 5) in children with chronic hepatitis C. The primary virologic remission, recovery of clinical and laboratory indicators, stabilization of fibroplastic changes and correction of hemodynamic disorders in the liver were observed in 57.1% of patients receiving the drug. This paper shows not only its positive effect on the dynamics of clinical symptoms of the disease, but, which is particularly significant, an improvement of blood flow in the liver and substantial increase in IFN level, especially α-IFN, after completion of treatment course, indicating that inhibition of HCV replication process [22, 23].

Under the guidance of Professor T.A. Kryuchko (2008) the controlled (under pre-approved by the State Expert Center MoH Ukraine Protocol) open-label study was conducted evaluating the efficacy and tolerability of Flavozid® (syrup) in children with chronic hepatitis B and chronic hepatitis C, which enrolled 71 patients aged 3 to 16 years. Flavozid® (syrup) is intended for use in pediatric patients from birth. The drug was administered at recommended dose and age against the background of basic therapy that included pathogenetic and symptomatic agents, hepato-protectors, antioxidants, chelators, drugs that improve the micro and lymphocirculation. In 27.7% of patients the replicative phase of infection was established, in 72.3% – integrative. In the integrated assessment of the clinical efficacy by determining the difference between the paired clinical indices before and after 3 months of treatment it was established that it amounted to 6.52, and in 6 months – 8.74, $p < 0.05$, indicating the high efficiency of the drug. This is also indicated by the results of the analysis of biochemical indicators of changes that reflect the functional state of the liver, including decreased levels of total, direct, and indirect bilirubin, ALT and AST in the serum. The fact of more rapid improvement in the condition of patients due to the ability of the drug Flavozid® (syrup) to stimulate the synthesis of endogenous a- and y-IFN was confirmed by data collected by the authors of their higher level in patients treated with this drug. Thus, patients treated with Flavozid® had α-IFN level

increased at the end of the therapy more than 3-fold, while in patients who received the basic therapy alone – 1.5-fold only. In general, the same pattern was obtained by analyzing the levels of y-IFN, and the viral load in patients of different groups. During the study, there was no objective or subjective symptoms of adverse reactions when using Flavozid® (syrup) in children of different age groups [24, 25].

Efficacy and safety of Proteflazid ® (drops) in pregnant women

Hepatitis B is a real threat to the life of the pregnant woman, fetus, and newborn. Deterioration in the 2nd half of pregnancy may be complicated by acute liver failure with encephalopathy and coma with a high mortality rate (mortality rate outside of pregnancy is 0.4-2% of pregnant women – 3-fold higher). HCV effect on gestational process in pregnancy is not established, but in some studies an increase was reported in the frequency of premature birth in HCV-positive women and 29% (general rate performance – 6-10% of all births). Treatment of viral hepatitis during gestation is associated with certain difficulties caused primarily by existing restrictions on use of a number of drugs in this period. The data on the efficiency and safety of Proteflazid® (drops) in the treatment of acute and chronic hepatitis B and C became the basis for the use of this drug in patients during pregnancy. The drug is not contraindicated for use in pregnant women since it has no teratogenic, mutagenic and embryotoxic action, so the use of antiviral and immunomodulatory Proteflazid® properties can ensure the reduction of the frequency of perinatal complications [26].

R.M. Mitsoda (2005-2007) analyzed the experience of Proteflazid® (drops) in pregnant women with hepatitis B and C, including in patients with active replication of pathogens. To correct the consequences caused by the HCV and HBV the drug was used (as having no teratogenicity) in addition to the basic therapy (glutargin, dufalak, enterosgel, ascorutinum) in more than 100 women. The group of women who received Proteflazid® showed almost 3-fold decrease in incidence of pathological blood loss during childbirth, lower frequency of anemia in pregnant women, 2.2-fold lower preterm delivery and significantly less inflammatory complications were observed in the postpartum period. This fact is explained by the immunomodulatory properties of Proteflazid® (drops): the drug administration improves the condition of both overall and local immunity. As a consequence an increase was observed in body's ability to resist the infectious agents in the postpartum period [27-29].

The resulting positive experience using Proteflazid® (drops) was the basis for its inclusion into regimen for prevention and treatment of obstetric complications in viral hepatitis.

Furthermore, according to R.M. Mitsoda (2006), the prescription of Proteflazid® (drops) for women with replicative activity of HBV or HCV in the period of gestation reduced the total number of asphyxia and hyporeflexia and acute ischemic lesions of the central nervous system in the early neonatal period of the fetus [30].

Similar results, which confirm the efficacy and safety of Proteflazid® (drops) in pregnant women, patients with acute and chronic hepatitis B,

were obtained by the experts of Research Institute of Obstetrics and Gynecology in Uzbekistan. Thus, Z.Sh. Zarzhova (2008) reported that the use of the drug in pregnant women reduces 2-fold the birth of children with pathology, improves the adaptation period and reduces the risk of transmission of HBV to children [31]. Furthermore, Z.Sh. Zaripova et al. (2008) showed that Proteflazid® (drops) in pregnant women with chronic hepatitis B leads to improved blood circulation; reduces degenerative changes in both the maternal and fetal placenta. Morphologic study showed the vasodilation and disappearance of phenomena in the edematous stroma placental tissue, which reduces the frequency of obstetric and perinatal pathology [32].

The issue of gestation course in women with a history of hepatitis B has been studied by experts of the P.L. Shupyk National Medical Academy of Postgraduate Education. N.V. Pekhnyo (2005), as previous authors, emphasizes that placental insufficiency occupies a leading place among the causes of the high rates of perinatal pathology of the fetus in pregnant women with hepatitis B. It is shown that the inclusion of Proteflazid ® (drops) into the conventional range of therapeutic and preventive agents for the correction of placental insufficiency provides a positive effect on the microcirculation in the body of pregnant women. The improvement is observed in vessels state of microcirculatory bloodstream in the course of pregnancy (reduction in the number of pathological deviations in all three links – vascular, intravascular and perivascular), as well as the course of pregnancy and childbirth, as evidenced by the decrease in the frequency of occurrence of the threat of termination of pregnancy from 22% in women, who were not taking the drug, up to 10% in the group treated with Proteflazid ®, anemia – from 38 to 18%, premature rupture of membranes – from 46 to 20%, intrauterine growth retardation – from 40 to 22%. The use of the drug also allowed preventing the development of decompensated placental insufficiency with the formation in 86% of cases and compensated at 14% – subcompensated form of placental insufficiency and a half (from 40 to 20%) to reduce the level of perinatal pathology of the fetus in pregnant women infected with HBV [33].

Summarizing the above, it should be noted that the data obtained in clinical practice, is consistent with the results of the preclinical stage studies, and confirm the specific activity of Proteflazid® against hepatitis B and C.

The table below shows the main results of 23 clinical observations.

The results of clinical studies on the efficacy and safety of Proteflazid® (drops), Flavozid® (syrup) in the treatment of hepatitis B and C in children, pregnant women and adults since 2000

№	Author, year, source	Number of patients: total enrolled in the study/receiving drug	The effectiveness of Proteflazid® / Flavozid®
In the treatment of hepatitis B and C in adults			
1	V.I. Matyash, 2000 [9]	45/30	Proteflazid® activity reduces the level of bilirubin from 293,7 ± 6,7 to 43,8 ± 4,5 mmol / l in the control (basic therapy) - to 278,8 ± 7,3 to 66,7 ± 3.9 mmol / l ($p < 0.05$). Normalization of bilirubin levels in the study group was observed in an average 8.2 ± 0.4 days earlier than in the controls, more rapidly decreased ($p < 0.05$) diluted ALT activity by week 4 - almost 1.5 ± 0,2 times (from 22,6 ± 1,4 to 3,2 ± 0,5 mmol / l h - study group, with a 21,1 ± 1,5 to 4,9 ± 0,6 mmol / h -l - control). Increased blood albumin from 47.1 + 0.7% to 52.9 ± 0.5% - study group, from 47.6 + 0.6 to 49.1 + 0.7% - control group. Elements of fibrosis in the liver in the study group were observed 2-fold less than in the controls; patients in the study group had 4-6 weeks earlier decrease in liver size to 2,8 ± 0,2 cm)
2	V. I. Matyash et al., 2002 [10]	45/25	Proteflazid® led within the month to a decrease of asthenia, vegetative and dyspepsia in 60.0% of patients (control - basic therapy in 45.0%). Recovery of satisfactory condition occurred in patients by an average of 7.1 days earlier than in the control group. Decreased hyperbilirubinemia level at the end of the 4th week was observed in 20.0% of patients (control 15.0%) on average 6.8-fold (control - 4.2 fold). At the 2nd month of treatment ALT decreased by almost 80% in the study group and only 60% in the control group (1.5 fold). Reduced proteinemii dispersion, reduced gamma fractions from 22.3 to 19.0% (control - from 21.9 to 20.3%), and increased albumin level - to 52.7% (control - 49, 1%). The phenomena of chronic, fibrotic degeneration of liver parenchyma occurred in 20.0% of patients in study group and 30.0% in the control group. Percussion decrease in liver boundaries was observed by 2.8 cm 6.9 days earlier in the study group
3	V. I. Matyash et al., 2002 [11]	85/85	Normalization of blood bilirubin during treatment with Proteflazid ® for 1 month in acute HVB was observed in 86.0% of patients, ALT - in 82.0%. The normalization of blood protein spectrum: dysproteinemia reduction, reduction of globulin, and in particular the gamma fractions from 22.3 ± 0.3 to 19.0 ± 0.2% in chronic flow, with 21.9 + 0.2 to 20.3 ± 0.3% - at an acute flow and accordingly, increased albumin - from 49.1 to 52.7% was observed in 76.0% of patients during the first month 2 - 90 ,4%. Reduction in the size of liver to 2,8 ± 0,2 cm and density for 1 month was observed in 98.8%. In acute course the phenomenon of fibrous degeneration of the liver parenchyma was observed only in 28.0% of patients. In 91.6% of patients a decrease of asthenia, vegetative and dyspepsia gastrointestinal tract dyskinesia was observed within 2-4 weeks,

4	B. M. Dikiy, 2003 [16]	37/17	After a week of Proteflazid ® therapy the patients in the main group showed decreased asthenic-vegetative, pain, dyspepsia and jaundice syndromes; subsequent reduction was reported in the size and density of the liver (in the control group - basic therapy - 10-12 days later). After 2 weeks of treatment a significant decrease was reported in cytolytic, mesenchymal-inflammatory syndromes, as well as the manifestations of cholestasis: 1.5-fold decrease in the activity of enzymes ALT, AST was recorded, thymol test - by 20-24%, blood gammaglobulin - 19-23%, total bilirubin - 1.-fold; conjugated diene content of malondialdehyde was significantly lower than in the control group - 2.46 ± 0.18 mmol / l and 19.26 ± 0.56 mmol / L, respectively, compared to 2.98 ± 0.22 mmol / l and 21.79 ± 0.74 mmol / l. Full normalization of biochemical parameters in the study group occurred in 84.4% of patients in the control group - in 68.7%
5	V. I. Matyash, 2003 [12]	120/80	Against the background of Proteflazid ® therapy for 3 months a regression of pathological process at complication of acute HBV was observed in 80.0% of patients and in 100% - when combined with Proteflazid® with plasmapheresis. The regression of the pathological process in chronic HBV when using the drug for 6 months was observed in 70.0% of patients and 90.0% - with the combination of Proteflazid® with plasmapheresis. Long-lasting therapeutic effect according to the normalization of transferase activity, lack of antigenemia and viremia in chronic HBV was observed in 55.0% of patients treated with monotherapy and in 75.0% - when combining Proteflazid® with plasmapheresis.
6	P. V. Nartov, O.V. Volobuyev, 2003 [15]	35/5	Patients treated with Proteflazid ®, compared with the control group (basic therapy), had improvement in a shorter period of common weakness, nausea, improved appetite, normalized liver sizes, and decreased yellowness of the skin. In patients treated with drug the changes in the level of bound and free bilirubin occurred much earlier. Thus, before hospital discharge, total bilirubin level was 27,1 ± 2,0 mmol / l, in patients in the control group - 48,6 ± 1,3 mmol / l. Reduced length of stay in hospital: in mild disease - 22,4 ± 0,4 (control - 27,9 ± 2,2), moderate disease - 28,1 ± 1,9 (control - 36,1 ± 3, 3)
7	O.L. Ivakhiv, 2004 [14]	56/30	Proteflazid® in mono- and combination therapy with cycloferon accelerated disappearance of clinical manifestations of the disease (nausea, poor appetite, general weakness), normalized liver size. CHC patients showed rapidly advancing biochemical remission: at discharge the bilirubin level in blood serum was significantly lower - 25.8 vs. 36.6 mmol / l in the control group (basic therapy); significant trend was noted towards normalization of ALT - 1.3 mmol / l and 1.5 mmol / L / h, respectively. Patients with chronic hepatitis B had positive dynamics of biochemical indices even more pronounced: serum bilirubin at discharge was 24.6 mmol / L vs. 34.8 mmol / l in the control group, ALT levels were significantly lower - 0.9 mmol / h-l to 1.4 mmol / l h. The reduced period of hospital stay was reported – average hospital stay was 16,7 ± 1,4 28,5 ± 2,6 vs. control group of patients

8	V. I. Matyash, 2004 [13]	40/20	The acute form of hepatitis B. Proteflazid ® within one month: reduces the severity of cytolytic and cholestatic syndromes, especially transferase activity, 43.7%, the number of total bilirubin - by 65.3%, reduces the size of the liver by 32.3%, general state improvement was observed in 88% of patients. Within 3 months: normalized parameters of transferase activity in 80% of patients and no cholestatic syndrome; decreased hepato-splenomegaly by 62%, recovered structure of the liver; decrease in number of patients with antigens in the blood and 25% viremia. CHB. Within one month: a decrease in the severity of cytolytic and cholestatic syndromes, especially transferase activity, by 37,8%, the amount of total bilirubin - more than 80% of normal levels, in 2/3 patients; decrease in hepato-and splenomegaly - 23.8%; moderate improvement in general condition, in particular of the gastrointestinal tract, reduction in severity of asthenovegetative syndrome. Within 6 months: decrease in severity and lack of cytolytic and cholestatic syndromes in 85% of patients, including reducing the level of circulating immune complexes in the blood of 65% of patients; sustainable improvement in general condition of patients: reduction of transferase activity by 81.5% (with normalization in 55% of patients); decrease of hepato-splenomegaly and 65% recovery of the liver structure
In the treatment of hepatitis B and C in children			
9	T.A. Kryuchko, I.N. Nesina, 2002 [18]	18/12	Proteflazid® improved clinical presentation within 11,3 ± 1,4 days in the control group (basic therapy) - 16,2 ± 1,7 days. The average duration of hyperbilirubinemia in infants of group 1 was 12,6 ± 1,12 days in the control - 18,3 ± 2,12 days. Along with the positive dynamics of laboratory data from the 4th week of taking the drug a significant reduction was observed in the size of the liver and spleen, no dyspeptic phenomena
10	T.A. Kryuchko, I.N. Nesina, Mohammed M.A. Abdalaal, 2005 [19]	57/27	While taking Proteflazid ®, even in the phase of the virus replication in moderate hyperenzymemia, intoxication symptoms (fatigue, decreased appetite, nausea) were observed only in 11.1% of patients and vascular "extra-hepatic" signs of splenomegaly - in 14.8%. Against the background of the drug therapy the occurrence of HBeAg was reported in 11% of patients and no HBV DNA in 30% of children with chronic hepatitis B, which may indicate a decrease in viremia. The level of IFN-a in comparison with the original data in children with chronic hepatitis B – 3-fold in patients with chronic hepatitis C – 4.2 fold
11	T.A. Kryuchko, Mohammed M.A. Abdalaal, 2006 [23]	54/24	The results of the serological examination of children after 6 months of treatment with Proteflazid ® demonstrated a significant decrease in anti-HCV IgM and reduction of HCV RNA, increased IFN-a. It demonstrated the normalization of peripheral hemodynamics: reduced resistance index and pulse index and an increase in blood flow and maximum flow velocity in the portal vein
12	I.N. Nesina, 2006 [21]	111/27	Proteflazid® reduces the intensity of clinical manifestations of CGD, which is accompanied by normalization of biochemical and virological parameters (remission was stable in 44.4% of patients), the positive dynamics of interferon status - an increase in IFN-a 3.5-fold and IFN- y – 1.4 fold as compared with parameters before treatment

13	Mohammed M.A. Abdalaal, 2006 [22]	108/24	Inclusion in the complex therapy of children with CHC Proteflazid® primary drug leads to remission in 57.1% of patients, restoration of clinical and laboratory parameters, signs of stabilization Fibroplastic changes and correction of hemodynamic disorders in the liver
14	T.A. Kryuchko, I.N. Nesina, 2006 [20]	47/27	While taking Proteflazid ® a regression was observed in symptoms of cytolytic syndrome: decrease in total bilirubin and its fractions as compared to parameters before treatment (36,5 ± 3,7 mmol / l vs. 24,0 ± 1,61 mmol / l, 14.35 ± 1.87 mmol / l vs. 8,1 ± 0,88 mmol / l; 22,15 ± 2,11 mmol / l vs. 15,9 ± 0,89 mmol / l); transaminases returned to normal (ALT 0,83 ± 0,09 mmol / l h against 0,6 ± 0,04 mmol / l h; AST 0,5 ± 0,06 mmol / l h v. 0.37 ± 0,03 mmol / l h). Therapy provides for liver function recovery, increases the secretion of bile and normalizes the lipid, carbohydrate and protein exchange
15	T.A. Kryuchko, I.N. Nesina, 2007 [24]	50/20	Flavozid® leads to clinical improvement in 80% of patients: normalization of pigment metabolism, decreased enzyme activity. Relief of pain occurred at an earlier date than in the comparison group (basic therapy). Studies of immune status in patients receiving the drug showed significant changes in lymphocyte subpopulations (increased number of initially reduced the number of T-helper cells, immunoregulatory index, and natural killer cells). Increased IFN-y concentration
16	T.A. Kryuchko, 2008 [25]	71/41	Flavozid® facilitates the liver function recovery. It increases secretion of bile and normalizes the lipid, carbohydrate and protein exchange. The concentration of IFN-a in children who received the drug, is 3.5-fold higher as compared with parameters before treatment and almost 2 fold the value in the comparison group (basic therapy). the concentration of IFN-y growth recorded 1.4 fold versus 1.1 in the control group regarding treatment parameters.
Treatment of hepatitis B in pregnant women			
17	R.M. Mitsoda, 2005 [27]	164/26	Proteflazid® in addition to the basic therapy in pregnant women with active HBV replication leads to lower rates of preterm and fast delivery, as well as reduces the amount of blood loss during childbirth
18	R.M. Mitsoda, 2006 [28]	99/35	In women with HBV replication Proteflazid ® drug reduces the frequency of hyperthermia (31.4 at 35.9 in the control group - basic therapy) and the total number of anemia of various severity (28.6 at 36.0 in the comparison group). The postpartum period in 51.4% of women was within normal, while this value was 32.8% in the comparison group
18	R.M. Mitsoda, 2006 [30]	99/49	Proteflazid ® in the treatment of HBV during pregnancy helps to 2-fold reduce the total number of asphyxia and 2.5 fold - acute ischemic CNS dysfunction relative to the comparison group (basic therapy)
20	R.M. Mitsoda, 2007 [29]	1158/42	Proteflazid® relative to the comparison group (basic therapy) can reduce the number of threatened miscarriages (28.4%); 5-fold reduce the rate of premature birth and number of fast delivery - 1.-fold; reduces the duration of periods of arid and blood loss, which ensures reduction in length of stay in a hospital (0.4 d.); newborns of women who received the drug are born half as often with the state of asphyxia and 2.5-fols less frequently with acute ischemic lesions of the central nervous system

21	N.V. Pekhnyo, 2005 [33]	150/50	Use of Proteflazid® for correction of placental insufficiency had a positive effect on the microcirculation in the body of pregnant women with a history of HBV. Compared with the control group (conventional therapy: vitamins, hepatoprotectors) an improvement was observed in microvascular course of pregnancy, decreased incidence of threatened miscarriages from 22 to 10%, anemia - from 38 to 18%, premature rupture of membranes - 46 to 20%, intrauterine growth retardation - from 40 to 22%; the drug allows doubled (from 40 to 20%) reducing the level of perinatal pathology in pregnant women with a history of HBV
22	Z.Sh/ Zarzhova, 2008 [31]	80/30	Proteflazid® had a favorable impact on the condition of the fetus in utero, CTG and Doppler improved to 2.5 times as compared to the comparison group (conventional therapy). It allows 2-fold reducing the birth of children with pathology, improving the period of adjustment and reducing the risk of infection transmission to a child.
23	Z.Sh. Zaripova, 2008 [32]	30/30	The results of morphometric studies have shown that during the treatment of chronic hepatitis B with Proteflazid® a marked improvement is observed in blood supply to the placental tissue, disappearance of edematous phenomena in the stroma. Vasodilation leads to increased oxygen supply of cell components, accompanied by a decrease in volume of syncytiotrophoblast 2 times and averaged 9,6 ± 1,7%. The fibrinoid area (4,8 ± 0,9%) and calcification (1,7 ± 0,3%) was significantly reduced by the action of the treatment.

In total, in 23 of the analyzed clinical observations 2756 patients were enrolled, of them – 1021 were treated with Proteflazid ® (drops) or Flavozid® (syrup). The authors of these studies reported the positive clinical and laboratory dynamics in patients taking drugs.

Particular attention is drawn to the use of Proteflazid® in pregnant women with hepatitis B or having a history of hepatitis in pregnancy. Proteflazid®, in addition to the basic treatment of hepatitis during pregnancy, can reduce the number of threatened miscarriages (28.4%); 5-fold reduce the rate of premature birth and 1.4-fold reduce the fast delivery; reduce the duration of periods of arid and blood loss, which ensures reduction in length of stay in a hospital; newborns of women who received the drug, are half as often born in a state of asphyxia and 2.5 times less frequently with acute ischemic lesions of the central nervous system. Against the background of the drug administration an improvement was reported in microvascular in pregnancy dynamics, reduced frequency of occurrence of the threatened miscarriages from 22 to 10%, anemia - from 38 to 18%, premature rupture of membranes - from 46 to 20%, intrauterine growth retardation - from 40 to 22%. The drug allows doubled (from 40 to 20%) reducing the level of perinatal pathology of the fetus in pregnant women infected with the HBV and risk of HBV transmission to children.

CONCLUSIONS

The preclinical studies have shown that Proteflazid ® effectively inhibits the expression of HCV RNA and BVDV as a surrogate reproduction both in monoinfection, and in mixed infection model (HIV + HCV). It is proved that the drug is an inducer of a- and y-IFN under in vitro and in vivo. In addition to antiviral activity Proteflazid® exhibits antioxidant and apoptosis modulating properties.

Systematic analysis of 23 independent clinical observations in more than 1000 patients showed a positive trend of treatment results in acute and chronic viral hepatitis using Proteflazid® (drops) and prepared on its basis Flavozid® (syrup), and confirmed the antiviral, immunocorrective, antioxidant, and apoptosis modulating properties in these drugs at the stage of pre-clinical studies.

Thus, Proteflazid® (drops) and Flavozid® (syrup) are safe and effective in a clinical setting for the treatment of acute and chronic hepatitis B and C in different groups of patients (adults, pregnant women, children of different age groups).

The results of successful clinical observations became the basis for the inclusion of Proteflazid® (drops) and Flavozid® (syrup) into the standard treatment regimen of viral hepatitis in various categories of patients as presented in a number of guidelines and information letters to the Ministry of Health of Ukraine [34-43].

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