Experimental application of pharmaceutical Gepon in combination with recombinant alpha-interferon in the patients with chronic hepatitis C

I.P. Gorbarets, N.V. Voronkova, T.V. Lopatina, V.N. Ivanovskaya, D.M. Braginsky

Clinical infectious hospital № 1, Moscow

The current treatment of chronic hepatitis C is based on the use of alpha-interferon in the form of monotherapy or in combination with ribavirin. The usual course of treatment includes injections of 3 million units of recombinant alpha-interferon, 3 times in the week over 12 months. With this interferon treatment it possible to impede the replication of hepatitis C virus (HCV), which is quantified by the reduction in the concentration of viral RNA or by its disappearance from the blood of the patients. According to the data produced by different authors, the prolonged therapy of alpha interferon can effectively reduce viral load on average in 30-50% of patients suffering from chronic hepatitis C infection [1-3].

Prolonged treatment with alpha interferon is accompanied by the development of the side effects: the overwhelming majority of patients present complaints of weakness, irritability, insomnia, head and muscular pains, and arthralgia (joint pain) in the course of treatment [5,12]. Frequent complications of interferon therapy include: anemia [4], neutropenia, thrombocytopenia [8], alopecia [5]. More rarely patients develop hypo-thyroidism [7], the pathologic damage of the capillaries [6], lupus [7], sarcoidosis [10] and bullous injuries of the skin [7], depression [10] and psychoses [9] and also the serious polyorgan toxic effects [13].

This serious side effect problem makes it urgent to search for new methods of the treatment for hepatitis C. In this work we report the successful combination therapy of recombinant alpha-interferon with the immunomodulator Gepon that reduced the side effects of interferon, without decreasing the effectiveness of the antiviral treatment. Gepon is a novel immunomodulator with anti-inflammatory activity, and possesses a wide spectrum of antiviral activity, including its ability to suppress the replication of HCV in both *in vitro* cell culture [14] and in patients suffering from hepatitis C disease [15].

This study accepted the participation by 21 patients (12 women, 9 men) sick with chronic hepatitis C, aged from 18 to 55 years. Patients were identified through the prolonged observation in The Gastroenterological Center in the Clinical Infectious Hospital № 1, Moscow. The diagnosis of chronic hepatitis C

was based on the presence of specific antibodies to HCV in the blood of the patients during at least 6 months. The presence of HCV RNA in the peripheral blood was determined by PCR (polymerase chain reaction), and in all patients in the trial the replication phase of HCV was documented. According to clinical data, and to the results of a biochemical study of the blood, in the majority of patients the moderate activity of chronic hepatitis C was established. All patients who participated in this investigation were treated with HGS recombinant alpha interferon at a dose of 3 millions units, 3 times a week. In addition to the background interferon therapy, 11 patients were treated with Gepon, 2mg orally (the preparation was dissolved in 5 ml of water, held 2-3 min in the mouth, then swallowed) once a day, daily during 3 months. Patients were divided into 2 groups:
1) patients, who obtained the combination of interferon antiviral therapy with Gepon (11 people);
2) patients, who obtained only the interferon antiviral therapy and no Gepon (10 people).

Treatment within the framework of the investigation continued for 3 months. Subsequently, the treatment with interferon was continued up to 1 year, even in the cases of the disappearance HCV RNA from the blood. For the duration of this study, all patients attended the designated doctor not less than 4 times - directly before beginning treatment, and 1, 2 and 3 months after the beginning of treatment. Within the same period, in addition to the clinical inspection, clinical and biochemical analyses of the blood were conducted, and also study HCV RNA by semi-quantitative PCR. The final estimation of efficacy of the treatment was conducted 3 months after the beginning of treatment. The criteria for efficacy were:
• the prevalence of complaints;
• the frequency of an improvement (or normalization) in the activity the hepatic enzymes al-AT, As-AT, gamma GT;
• the frequency of improvement in the clinical indices of the blood;
• the results of the PCR analysis of HCV RNA (viral replication)

Statistical processing of the results of treatment was conducted with the use of a computer program of ‘Statgraphics’

Initial clinical status of the patients (before treatment)

Patients in both treatment and control groups prior to the beginning of treatment were similar in the presence and manifestation of the symptoms characteristic for the disease of the hepato-biliary zone. Complaints of pain in by right subcostal area was presented in 16 out of 21 (76%) patients. Bitterness in the mouth was perceived by 15 patients (71%). Express weakness, a sign of general asthenovegetative syndrome, appeared distinctly in 11 (52%) patients.

With the inspection only one of the subjects had established jaundice of the skin and sclera. Hepatomegalia and splenomegalia occurred in 20 (95%) and 17 (81%) respectively of the patients of both groups. Diffuse changes in parenchyma of the liver were registered with UZI in 20 of 21 patients (95%).

According to the UZI data, one of patients had reactive pancreatitis. In certain cases it was noted the pathology of gall bladder, in particular, thickening the walls of gall bladder in 9 people, the bend of bilious duct in 6 people, and concretements in the gall bladder in 1 patients.

Dynamics of the clinical manifestations in the course of treatment

During the course of treatment, the presence and the manifestation of dyspeptic phenomena were evaluated (pain in the right subcostal area, bitterness in the mouth), asthenovegetative symptoms (weakness, irritability) and arthralgia. The fraction of patients with the presence of the corresponding index in the investigated groups is represented in figures 1 and 2.
In the group of patients who obtained only the interferon therapy for 3 months of treatment, the frequency of complaints of expressed general weakness grew from 50% to 80%, irritability - from 0 to 100%, arthralgia - with 0 to 90% (Fig. 1).

Fig. 1. Dynamics of the sign of general intoxication of the HCV patients during the course of treatment interferon alone or in combination with Gepon (the percentage of patients with the presence of this symptom is indicated)

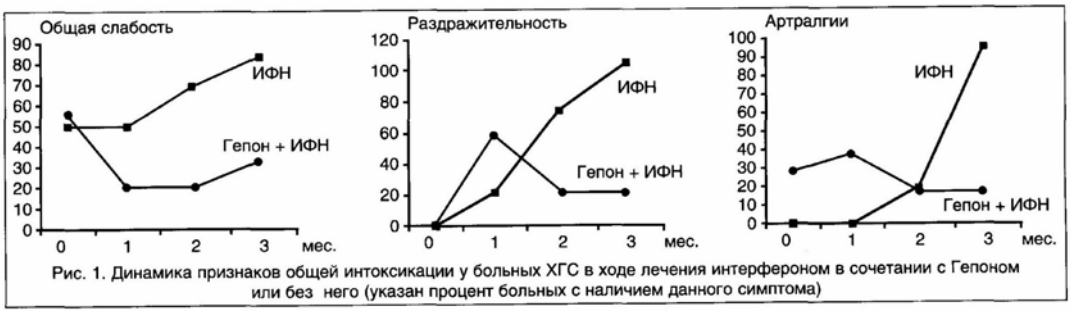


Рис. 1. Динамика признаков общей интоксикации у больных ХГС в ходе лечения интерфероном в сочетании с Гепоном или без него (указан процент больных с наличием данного симптома)

The related deterioration in the health of patients in the course of interferon therapy can be explained by the widely known side-effects of all preparations of interferon [5,12].

In the group of patients, who obtained interferon in combination with Gepon, the dynamics of the interferon side effects did not tend to increase. On the contrary, the frequency of complaints of the general weakness was reduced from 54% to 18% ($p<0.05$), arthralgia - from 27% to 18% ($p<0.1$). Irritability in the first month of treatment was noted in 54% of patients, in the subsequent months of treatment this index was reduced to 18% ($r<p<0.05$, Fig. 1). The obtained results testify ***the positive influence of Gepon on the adverse reactions to interferon therapy in HCV patients.***

In the majority of patients of both groups prior to the beginning of treatment, symptoms associated with liver disease was present. Thus, 70-80% of the patients noted pains in the right subcostal area and perceived bitterness in the mouth. In the course of 3 months of treatment with interferon only, the

patients of control group, the frequency of complaints of the pain in the right subcostal area and the bitterness in the mouth did not change. In the group of patients, who obtained treatment with interferon in combination with Gepon, the frequency of complaints of the pain in the right subcostal area was reduced from 80% to 18% ($p<0.05$). After 2-3 months of treatment, the bitterness in the mouth was perceived in only 9% of the treatment group against 64% in the controls ($p<0.05$) in comparison to the percent of patients before beginning treatment (Fig. 2).

Fig. 2. Dynamics of the symptoms associated with the disease of the liver, in the course of treatment with interferon alone or in combination with Gepon (the percentage of patients with the presence of this symptom is indicated)

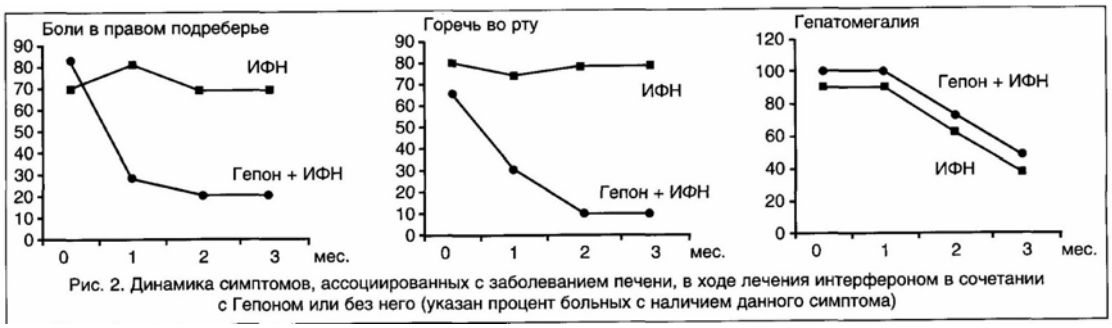


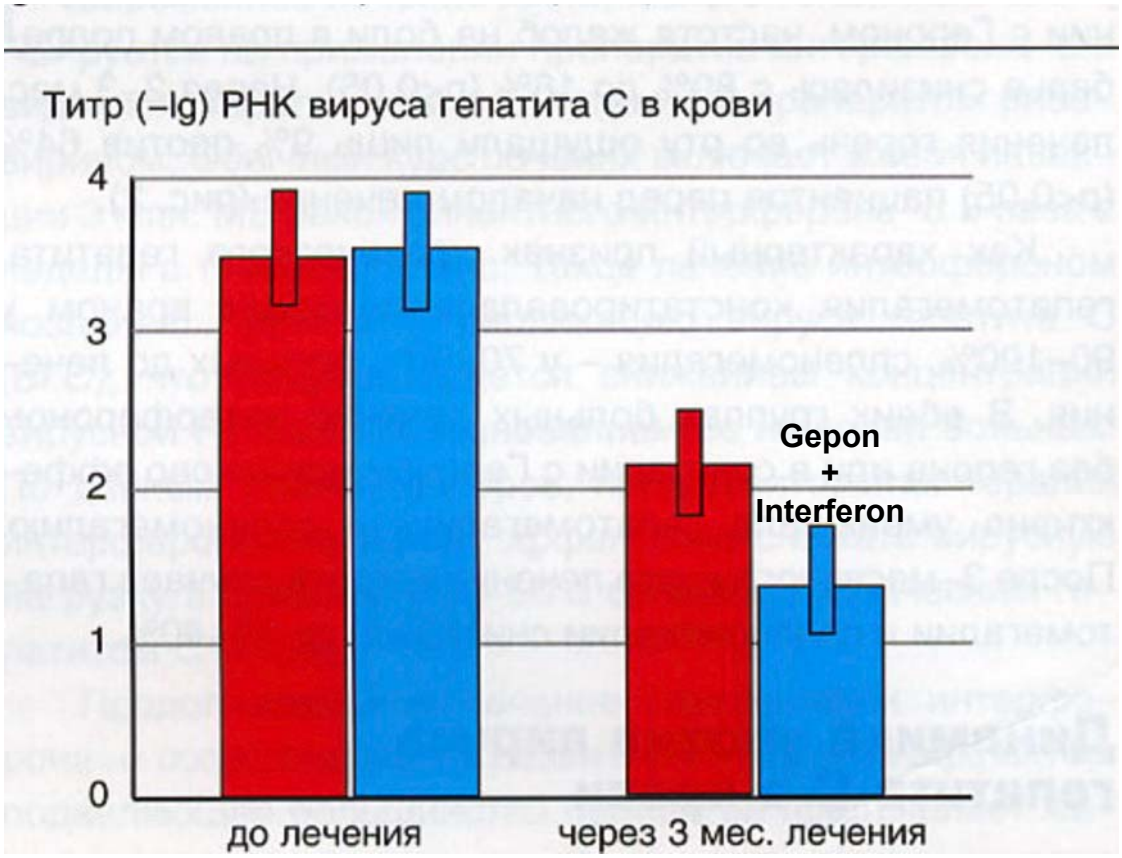
Рис. 2. Динамика симптомов, ассоциированных с заболеванием печени, в ходе лечения интерфероном в сочетании с Гепоном или без него (указан процент больных с наличием данного симптома)

The characteristic features of chronic hepatitis, such as hepatomegaly was noted by the doctor in attendance in 90-100% of patients and splenomegalia in 70-90% of patients before the treatment. In both groups of patients the treatment with interferon without Gepon or in combination with Gepon equally effectively decreased the hepatomegaly and splenomegalia. After the 3-monthly course of treatment the frequency of the cases of hepatomegaly and splenomegalia was reduced to 30-40%.

Dynamics of the level of the hepatitis C virus in the blood

In all 21 patients investigated by us, a semi-quantitative PCR estimation of the level HCV RNA in the blood was performed. Before treatment, in the group of patients who obtained only interferon therapy, the titre of HCV RNA varied from 10^3 to 10^4 and in 7 (70%) of 10 people after three 3 months from the beginning of treatment, the titres of HCV RNA were reliably lower than the initial levels. HCV RNA ceased to be determined in 2 (20%) of 10 patients. In the interferon control group, the titre of HCV RNA was reduced by 1.3 log, i.e about 20 fold (Fig. 3). In one patient in the control group, the level of virus increased 10 times during the course of interferon treatment

Fig. 3. Reduction in the viral load as a result the 3- monthly treatment of HCV patients with interferon alone or in combination with Gepon.



In the group of patients, who received Gepon in combination with interferon, the suppression on the replication of virus was more effective than in the control group, which obtained only interferon. 10 (91%) out of 11 patients had a reliable reduction in the concentration HCV RNA in the blood. The average titre of HCV RNA in the group was reduced by 2,2 log, i.e., 158 times. In 4 (36%) out of 11 patients HCV RNA ceased to be detected in the blood. Not one patient of the treatment group registered an increase in the viral load.

Biochemical indices of the blood

In the patients sick with the chronic hepatitis C, of those who participated in the investigation, it was noted a moderate increase in the level of al-AT and As-AT in the blood, that indicates the destructive inflammation of the hepatocytes. In both investigated groups after only 1 month of treatment with interferon alone or in the combination Gepon, a significant decrease in the level of al-AT and As-AT in the blood occurred, which continued during the course of the subsequent 2 and 3 months (the period of observation). However, it was noted a tendency toward a deeper reduction in as-AT and Al-AT during the use of Gepon (Fig. 4).

Fig. 4. Dynamics of aminotransferases (AT) and bilirubin in the blood of HCV patients as a result the 3- month treatment of HCV patients with interferon alone or in combination with Gepon

Рис. 4. Динамика аминотрансфераз и билирубина в крови больных ХГС в процессе 3–х месячного лечения интерфероном в сочетании с Гепоном или без него

The average values of general bilirubin in both groups of patients were in the region of upper boundary of standard. In the course of treatment the level of general bilirubin of the blood decreased by 15-20% to normal values. The dynamics of bilirubin in both groups is represented in figure 4, the reliable differences between the groups it is not observed.

Hematologic indices

The side effects of the prolonged therapy by the preparations of recombinant interferon appear in changes in the indices of the blood. In the control group of patients who obtained treatment with interferon alone, during the course of conducting the present investigation, it was observed that a significant reduction of the maintenance of hemoglobin, number of thrombocytes and especially quantity of ripe neutrophilic granulocytes occured, (Fig. 5), and simultaneously there was an increase of the content of lymphocytes, eosinophils (Fig. 6) and macrophages/monocytes (Fig. 7).

Fig. 5. Dynamics of hematologic indices (hemoglobin, neutrophiles, thrombocytes) as a result the 3- month treatment of HCV patients with interferon alone or in combination with Gepon

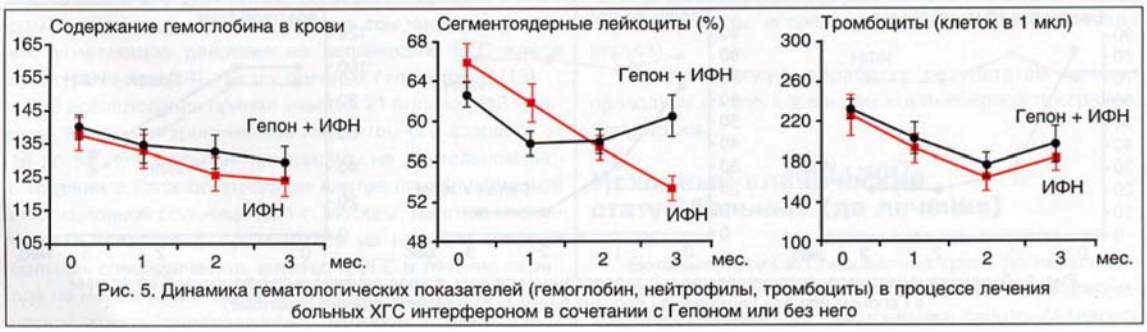


Рис. 5. Динамика гематологических показателей (гемоглобин, нейтрофилы, тромбоциты) в процессе лечения больных ХГС интерфероном в сочетании с Гепоном или без него

The combination of Gepon with interferon prevented the majority of the pathologic changes occuring. In patients, who obtained interferon in the combination Gepon, neutrophilis, granulocytes, eosinophils, lymphocytes and macrophages/monocytes remained at normal values of during the 3 months of treatment (Fig. 5-7).

Fig. 6 dynamics of hematologic indices (lymphocytes, the eosinophils as a result the 3- month treatment of HCV patients with interferon alone or in combination with Gepon

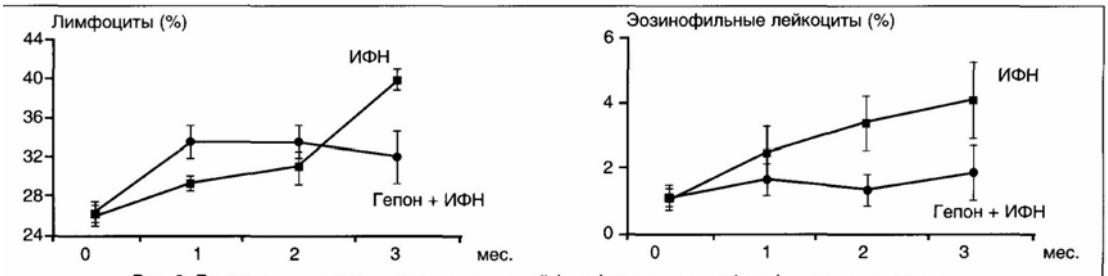


Рис. 6. Динамика гематологических показателей (лимфоциты, эозинофилы) в процессе лечения больных ХГС интерфероном в сочетании с Гепоном или без него

Fig. 7. Dynamics Macrophages/monocytes as a result the 3- month treatment of HCV patients with interferon alone or in combination with Gepon.

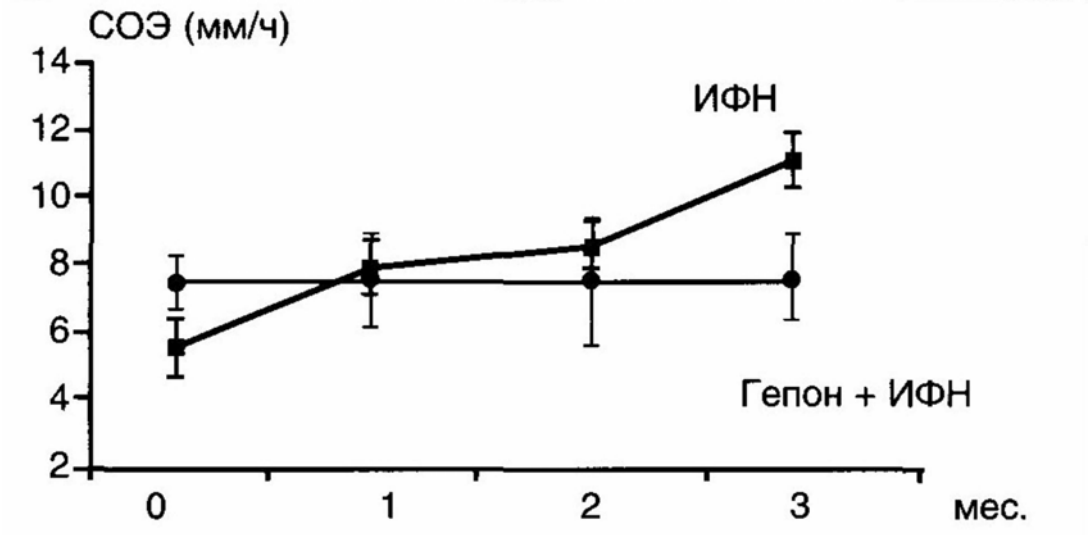


Рис. 7. Динамика СОЭ в процессе лечения больных ХГС интерфероном в сочетании с Гепоном или без него

Conclusion

The results present above are the first published experiment of the combined application of recombinant interferon with the immunomodulator Gepon in the treatment of the chronic hepatitis C. The results testify that **the combination of these two preparations leads to an improvement in the quality of the life of patients,** who obtain interferon therapy, decreasing the frequency of such symptoms as weakness, irritability, arthralgia, pain in the right subcostal area, bitterness in the mouth for the 3 months of treatment.

The comparison of the level of viremia in the group of patients, who obtained monotherapy interferon (reaferon), with the group who were treated by the combination of Gepon plus alpha interferon (reaferon) revealed that the average reduction in the concentration HCV RNA through the 3 months of treatment with the combined therapy, was 7 times higher. The disappearance HCV RNA from the blood was more frequently observed with the Gepon combined therapy.

In the clinical practice it is known that the elimination HCV RNA in 3 months of treatment by the preparations of interferon is the predictor of positive stable response to chronic hepatitis C. The data obtained in the present investigation makes it possible to assume that the efficacy of the combination of alpha interferon with Gepon is higher than mono-therapy for patients sick with chronic hepatitis C.

Literature

1. Jacobson K.R.; K. Murray: A.Zellos; K.B.Schwarz. An analysis of published trials of interferon monotherapy in children with chronic hepatitis C J. pediatric gastroenterology and nutrition (United States).- Jan 2002.- 34:(1).- p.52-8.
2. Nguyen M H;TL. Wright. Therapeutic advances in the management of hepatitis B and hepatitis C. Current Opinion in Infectious Diseases (United States).-Oct 2001. - 14:(5). -p.593-601.
3. Wright M: J. Main; H.C. Thomas. Treatment of chronic viral hepatitis C Antiviral Chemistry & Chemotherapy (England).- Jul 2001.- 12:(4).- p.201-12.
4. Gergely A.E.; P. Lafarge; I. Fouchard-Hubert; F. Lunel-Fabiani. Treatment of ribavirin/interferon-induced anemia with erythropoietin in patients with hepatitis C, Hepatology (Baltimore. Md.. United States).- May 2002.- 35:(5).-p1281-2.
5. Zucker D M: S W. Miller. Assessment of side effects in patients with chronic hepatitis C receiving combination therapy, Gastroenterology nursing (the official journal of the Society ot Gastroenterology Nurses and Associates, United States). Jul-Aug 2001. 24:(4).-p.192-6.

6. Yamamoto K.; M. Mizuno: T. Tsuji; T. Amano Capillary leak syndrome after interferon treatment for chronic hepatitis C, Archives of internal medicine (United States). - Feb 25. -2002.-162:(4).-p481-2.
7. Pouthier D.; F. Theissen; ft-/.. Humbel. Lupus syndrome, hypothyroidism and bullous skin lesions after interferon alpha therapy for hepatitis C in a haemodialysis patients, Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association (England).-Jan 2002.~17:(1).-p.174.
8. Sagir A.; M. Wettstein; T. Heintges: D. Haussinger. Autoimmune thrombocytopenia induced by PEG-IFN-alpha2b plus ribavirin in hepatitis C Digestive diseases and sciences (United States). -Mar 2002.- 47:(3).- p.562-3.
9. Castera L; F. Zigante; A. Bastie; C. Buffet; D. Dhumeaux; P. Hardy, Incidence of interferon alpha-induced depression in patients with chronic hepatitis C, Hepatology (Baltimore, Md., United States).-Apr 2002.-35:(4).- p. 978-9.
10. Wendling Jeanne; V. Descamps; M. Grossin: P.Marcellin; P. Le Bozec: S. Belaich; B.Crick. Sarcoidosis during combined interferon alpha and ribavirin therapy in 2 patients with chronic hepatitis C, Archives of dermatology (United States).-Apr 2002.-138:(4). p.546-7.
11. Stransky J; J. Skrivankova. Serious side-effects of interferon alpha and ribavirin combination therapy in patients with chronic hepatitis C, Vnitrni lekarstvi (Czech Republic). Jan 2002, 48:(1).- p.56-9 (in English).
12. Carreno V. Present treatment expectations and risks of chronic hepatitis C, Clinical Microbiology and Infectious Diseases (France).- Feb 2002.-8:(2).- p74-9.
13. Lafeulliade A., G. Hittinger, S. Chadapaud. Increased mitochondrial toxicity with interferon/ribavirin in HIV/HCV coinfection. Lancet.- 2001.-v.357.- Jan 27.-p.280-281.
14. Ataullakhanov R.I., R.D.Holms. A.V.Katlinsky, P.G Deryabin, A N.Narovlyanskiy, M.V.Mezentseva, F.I.Ershov. The immunomodulator Gepon suppresses the replication of HCV virus in human hepatocyte cell culture Antibiotics and Chemotherapy -2002. - vol. 47-№8.-S.9-11.
15. Cherednichenkos T.V., V.F.Uchaykin. G.V.Chaplygina, G.M.Kurbanova. Gepon: new effective treatment of HCV, Prescribing Doctor -2003.-№3.-S.82-83.