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Double Filtration Plasmapheresis in Treatment of Patients With Co-Infection of Hepatitis C and Human Immunodeficiency Virus

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Abstract: One of the main causes of mortality of human immunodeficiency virus (HIV)-infected patients are complications of chronic hepatitis C virus (HCV). Combining drug therapy for HCV with double filtration plasmapheresis (DFPP) has significantly increased the effectiveness of treatment for these patients. However, there are no data on the use of this method for the treatment of patients co-infected with HIV and HCV. We demonstrated that positive clinical effect in the treatment of HCV patients by DFPP (previously demonstrated) is also achieved in the treatment of HIV infected patients, co-infected with HCV. The obtained efficiency of 62.5% is almost two times higher than the predicted treatment efficiency. We can conclude that the complex therapy of hepatitis C, including DFPP and medication by PEG-IFN + RBV is an effective and safe approach for the treatment of HCV in patients co-infected with HCV and HIV. **Key Words:** Co-infection, Hepatitis C, Human immunodeficiency virus, Plasmapheresis.

The appearance of highly effective antiretroviral drugs and successes in preventing the majority of opportunistic infections have led to a significant increase in the survival rates and in the quality of life of HIV-infected patients. Currently, one of the main causes of mortality of these patients are complications of chronic hepatitis C virus (HCV) (1,2).

There is some evidence that the concentration of the hepatitis C virus in the blood plasma and liver tissue is higher in patients who are infected both by HIV and HCV, than in those without co-infection. These patients also have more pronounced inflammatory processes in the liver. Fibrosis in these patients also develops faster (2). Significant progress has been made in the treatment of HCV by therapy with pegylated interferon and ribavirin (PEG-IFN +RBV). However, for some patients with HCV (genotype 1), the effectiveness of such treatment is still rather low (from 34 to 42%) (3).

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Combining drug therapy for HCV with double filtration plasmapheresis (DFPP) has significantly increased the effectiveness of treatment for these patients (4). It has been shown that if three to five DFPP procedures are carried out daily at the beginning of medical treatment, it will lead to a sustained virologic response in 62% of patients, which is significantly more effective than the treatment with PEG-IFN + RBV only. This method is called VRAD therapy – Virus Removal and Eradication by DFPP. Thus, the mechanical removal of HCV viral particles and immune complexes that contain those viral particles from the blood during the DFPP procedure give the significant clinical benefit.

However, there are no data on the use of this method for the treatment of patients co-infected with HIV and HCV. The only case described is an application of DFPP for the treatment of the HIV infection complicated by Henoch-Schonlein purpura nephritis and nephrotic syndrome (5). The safety of DFPP application and the effect of this procedure in HIV patients to date have not been investigated. The aim of this study was to evaluate the efficacy and safety of DFPP in combination with PEG-IFN and RBV in patients co-infected with HCV and HIV.

PATIENTS AND METHODS

Patients

The study included nine patients co-infected with HIV and HCV genotype 1, mean age 39.5 ± 10.8 years, including four women and five men. Seven patients prior to and during the study received antiretroviral therapy (HAART) as carriers of the HIV infection. Before the study all patients received a course of antiviral drug therapy for HCV: patients received PEG-IFN + RBV. At the time of inclusion in the study, two patients did not respond to PEG-IFN + RBV therapy, seven had HCV recurrence after the completion of the therapy.

Patient #4 began receiving HAART therapy during the study. The reason was the increase in HIV viremia after 24 weeks of the DFPP PEG-IFN + RBV therapy. Changes in the level of CD4+ lymphocytes were not observed in this patient. Patient # 8 at the start of treatment and throughout all research did not receive HAART therapy, because the level of HIV viremia and CD4+ lymphocyte quantity did not require its use.

Viremia (HCV) in all patients was higher than 4×10^6 IU/mL. HIV RNA at baseline was determined in two patients (patients #4 and #8) who were not receiving HAART, the viral load in these patients was 0.24×10^5 and 1.6×10^5 copies/mL, respectively. The liver damage was compensated (Class A in the clinical classification of Child–Pugh, F0–F1 in the Metavir score). Prior to the study, over the course of 6 months patients did not use antitumor or immunomodulating drugs. There were no drug addicts among them.

The number of neutrophils in all patients at baseline exceeded 1.5×10^9/L, platelets exceeded 150×10^9/L, and the hemoglobin concentration was above 120 g/L.

Drug therapy

Drug therapy included PEG-IFN (PEG-IFN-α2a 180 μg per week, or PEG-IFN-α2b 1.5 μg/kg of body weight per week) and RBV 15 mg/kg of body weight per day – 2 twice a day for 48 weeks.

DFPP treatment

Three DFPP procedures with intervals of 1 day were carried out at the beginning of treatment (0 week), during the 1st week to the 48th the causal course of drug therapy was conducted, and during the period from 49th to 72th week, the observation was carried out.

DFPP was performed with the help of devices for plasmapheresis "GEMMA" (ZAO "Plasmofilter", Saint-Petersburg, Russia) and two filters. Membrane plasma filter PFM-500 with pore size 500 nm (ZAO "Plasmofilter", Saint-Petersburg, Russia) was used as the first filter, and plasma fractionator Evaflux 5A20 (Kawasumi Laboratories, Tokyo, Japan) was used as the second one. The volume of the treated plasma during DFPP procedure was 120% of the circulating plasma volume. Anticoagulation was performed with heparin and a 4% sodium citrate. Bolus injection of heparin was used prior to the procedure in the amount of 70–100 U/kg of body weight, 4% sodium citrate was infused continuously during the procedure at a ratio of 1:15–1:20 to the blood flow.

Virology

The concentration of HIV and HCV viruses in the patient's blood was determined with real-time PCR (Roche Diagnostics, Basel, Switzerland and Abbott Labs, Lake Bluff, IL, USA).

The viral load of HIV and HCV were determined with the automated systems Abbott m2000 RealTime System and the Cobas AmpliPrep/Cobas TaqMan. For the determination of the HIV viral load the Abbott RealTime HIV 1 test system was used (the lower range limit was of 40 copies/mL), and Cobas AmpliPrep/Cobas TaqMan HIV 1 Test, v.2.0 (the lower range limit was 20 copies/mL). HCV viral load was determined with Abbott RealTime HCV test system (the lower range limit was 12 IU/mL) and Cobas AmpliPrep/Cobas TaqMan HCV Quantitative Test, v.2.0 (lower range limit was 15 IU/mL).

Virologic response was classified in accordance with EASL (European Association for the Study of the Liver): rapid virological response (RVR) – response within 4 weeks after the start of the drug treatment, complete early virologic response (cEVR) – response within 12 weeks after the start of the drug treatment, sustained virologic response (SVR) – response within 24 and 48 weeks after the end of the drug treatment.

Data collection and measurements

Before the course as well as after the 1st and 3st procedure (samples were taken the next day after the treatment), the following parameters were determined: the complete blood count, CD4+ cells, the concentration of total protein, albumin, immunoglobulin A, M, G, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), and fibrinogen. The concentration of low density lipoprotein cholesterol (LDL-C) was calculated with the Friedewald formula.

For biochemical studies the automatic biochemical analyzer Cobas 6000 module 501 was used (Roche Diagnostics). Immunological tests (determination of CD3, CD4, CD8) were performed using four-color antibodies (FITC, PE, APC, VBR) with flow cytometry (BD FACS Canto II and BD FACS Calibur,

Becton Dickinson, Franklin Lakes, NJ, USA). Hematological studies were performed with Sysmex XT-4000i Automated Hematology Analyzer. The automatic analyzer Architect i 2000 SR (Abbott Diagnostics) was used for serological studies.

Statistical analysis

Results were expressed as mean ± SD and analyzed using Student's *t*-test for paired samples. Statistical significance was assumed for $P < 0.05$.

RESULTS

DFPP was performed in nine patients co-infected with HIV and HCV (genotype 1). Seven patients prior to and during the study received antiretroviral therapy (HAART) as carriers of the HIV infection. The overall follow up period was 73 weeks. During the treatment, patient #1 was excluded from the study because of neutropenia that developed in the 4th week of drug therapy (PEG-IFN therapy leads to the termination of treatment due to neutropenia in approximately 4% of patients).

The study of blood from six patients receiving HAART showed that the level of red blood cells and platelets in the course of treatment did not change significantly from baseline. The maximum decrease was observed at 12 and 48 weeks of treatment (Table 1).

The reduction of neutrophils and lymphocytes during the complex treatment, that included DFPP courses and PEG-IFN + RBV therapy, was significant (Table 1). The minimum level of neutrophils was observed in the 24th and 48th week of treatment (47% and 41% of the initial, respectively); minimum lymphocytes were observed the 36th and 48th week of treatment (58% and 60%, respectively, of the initial).

The absolute number of CD4+ lymphocytes in patients receiving HAART together with DFPP and PEG-IFN + RBV was decreased. The maximum decrease was observed at week 12 of treatment (an average of 25%), that is associated with the reduction in the total number of lymphocytes.

Figure 1 illustrates the change in the level of CD4+ lymphocytes in the course of the study, in patients treated with HAART ($N = 6$) and in patients that were not receiving HAART (patients #8 and #4). The dynamics of CD4+ lymphocytes in patient #8 completely matches with changes in the level of CD4+ lymphocytes in patients that have received HAART. The number of CD4+ lymphocytes in the patient #4 decreased in 24 weeks to 250 cells/μL, which was an indication for the start of HAART administration in this patient. After the start of HAART, the number of CD4+ lymphocytes began to increase (Fig. 1).

After the course of three DFPP treatments the concentration of LDL-C (73%), IgM (62%), total cholesterol (55%), triglycerides (54%) and HDL-C (39%) reduced significantly in the blood plasma of all the patients. Also, there was a statistically significant decrease in the concentration of total protein, albumin, IgG (Table 2).

For safety evaluation during the DFPP treatment, the platelet count and the concentration of fibrinogen were determined. After the DFPP procedure the platelet count did not decrease significantly, the concentration of fibrinogen reduced from 3.26 ± 0.32 to 1.82 ± 0.2g/L. However, on the next day after DFPP the fibrinogen partly restored (up to 2.54 g/L on average). Four weeks after the completion of the course DFPP it was 88% of baseline (average of 2.87 g/L), despite the presence of hepatic dysfunction in patients.

The study of the dynamics of HCV RNA content and HIV RNA in the blood plasma during the DFPP procedure showed the complete removal of viruses HCV (Fig. 2) and HIV (Fig. 3).

After three DFPP treatments, the viral load of HCV RNA in 6 of 8 patients decreased on average 80% (40 to 97%) relative to the level before the treatment (Fig. 4). Subsequently, the rapid virologic response (RVR) was observed in 2 of 6 patients (#6

TABLE 1. *Changes in the blood cells of patients coinfected with hepatitis C virus (HCV) and HIV, treated with HAART during the study ($N = 6$)*

Indicator	Before treatment	Duration of treatment (weeks)				
		4	12	24	36	48
Red blood cells, $\times 10^{12}$/L	4.4±0.4	3.6±0.6	3.5±0.4	4.0±0.2	3.6±0.2	3.5±0.2
Platelets, $\times 10^{9}$/L	155±6	165±16.0	162±47	175±12	165±9	154±4
Neutrophils, $\times 10^{9}$/L	2.7±0.9	1.4±0.2*	1.4±0.6*	1.3±0.3**	1.5±0.9*	1.1±0.5**
Lymphocytes, $\times 10^{9}$/L	1.7±0.2	1.3±0.4*	1.2±0.3*	1.2±0.4*	1.0±0.3**	1.0±0.3**
CD4+, cells /μL	514±64	396±69	383±30*	442±75	410±36	394±58

*$P < 0.05$. **$P < 0.02$. Data are presented as mean ± SD.

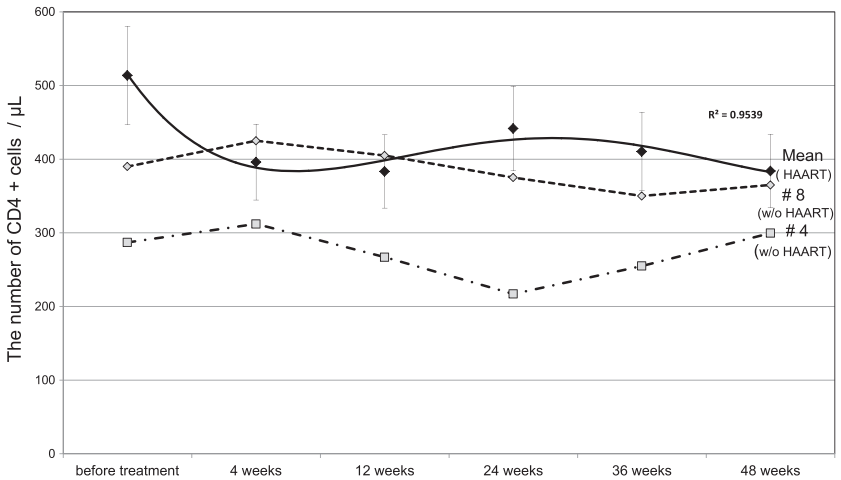

FIG. 1. The dynamics of CD4+ lymphocytes in patients co-infected with hepatitis C virus (HCV) and HIV during the treatment by double filtration plasmapheresis (DFPP) and PEG-IFN + RBV. ♦, patients receiving HAART ($N = 6$); ■, patient #4; ♦, patient #8 who did not receive HAART during the study.

TABLE 2. *Dynamics of blood plasma parameters during the course of double filtration plasmapheresis (DFPP)*

Indicator	*N*	Before DFPP treatment	After the 1st DFPP procedure	After three DFPP procedures	
				Absolute values	Reduction
Total protein, g/L	16	69.8 ± 1.7	52.2 ± 4.5*	54.9 ± 1.6*	21%
Albumin, g/L	16	42.3 ± 1.0	33.2 ± 2.7*	35.2 ± 1.1*	17%
Total cholesterol, mM/L	13	4.43 ± 0.21	2.29 ± 0.95*	2.00 ± 0.17*	**55**%
Triglycerides, mM/L	12	1.45 ± 0.28	0.53 ± 0.20*	0.67 ± 0.11*	**54**%
Cholesterol HDL, mM/L	10	1.27 ± 0.20	0.99 ± 0.10	0.78 ± 0.07*	39%
Cholesterol LDL, mM/L	10	2.73 ± 0.28	1.12 ± 0.17*	0.73 ± 0.07*	**73**%
Ig M, g/L	13	1.43 ± 0.14	0.96 ± 0.17	0.55 ± 0.15*	**62**%
Ig G, g/L	13	26.9 ± 1.3	20.5 ± 2.4	20.6 ± 1.7*	**23**%
Ig A, g/L	13	1.89 ± 0.24	1.32 ± 0.99	1.21 ± 0.21	**36**%

*$P < 0.05$. **$P < 0.02$. Data are presented as mean ± SD. Reduction in more than 50% was marked as bold.

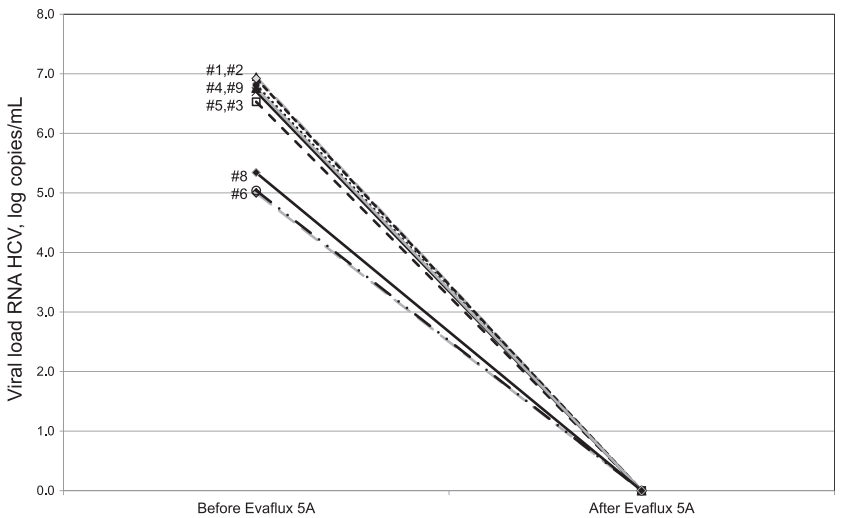

FIG. 2. The dynamics of viral load of HCV RNA during the double filtration plasmapheresis (DFPP) procedure in plasma before and after plasma fractionator Evaflux 5A (patients #2–8).

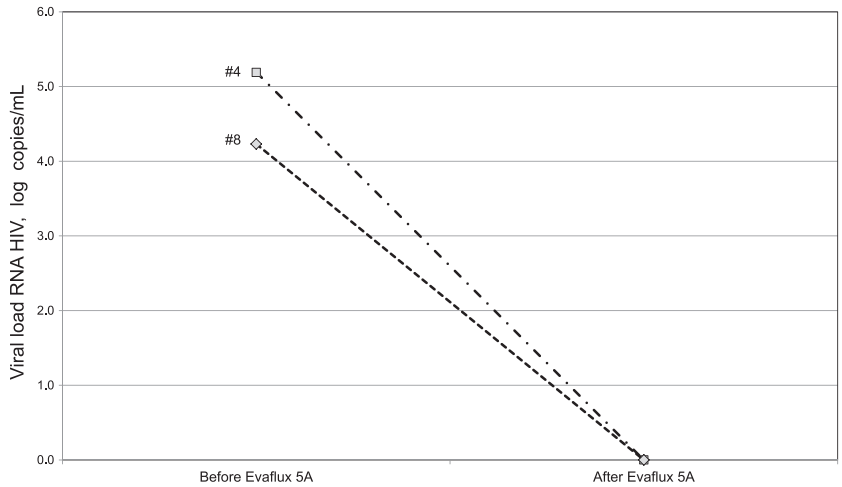

FIG. 3. The dynamics of viral load of HIV RNA during the double filtration plasmapheresis (DFPP) procedure in plasma before and after plasma fractionator Evaflux 5A in patients #4 and #8.

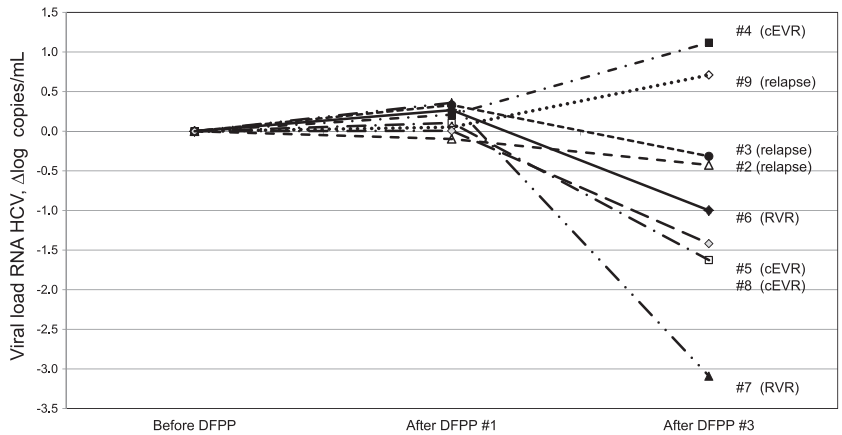

FIG. 4. Dynamics of hepatitis C virus (HCV) viral load during the course of the three during the double filtration plasmapheresis (DFPP) procedures in patients with different responses to the treatment (DFPP + PEG-IFN-RBV). (Patients who responded to the treatment at the 4th week. (RVR): # 6, 7; patients who responded to treatment at the 8th week. (cEVR): # 4, 5, 8; patients with a relapse during or after therapy: #2, 3, 9).

and #7), the complete early virologic response (cEVR) occurred in two patients (#5 and #8), two patients (#2 and #3) – had the recurrence of HCV.

In two patients the viral load increased during the course of DFPP treatment (#4 and #9). The viral load in the patient # 9 had increased seven times. The virologic response to treatment in this patient was subsequently fast (RVR); however, at 4 weeks after the end of treatment (52 weeks from the beginning of treatment) recurrence occurred. The viral load in the patient #4 after the course DFPP increased 11 times. Subsequently, the early (cEVR) and stable (SVR) virologic response was obtained in this patient.

We also monitored the dynamics of HIV viral load during the course of treatment in two patients who did not receive HAART. The concentration of HIV in the blood plasma of patient #4 after the 1st DFPP procedure increased by 33%, and after the third it decreased by 43% compared to before treatment level. The HIV viral load above 10^6 took place at the 24^{th} week of the study in this patient, so that the administration of HAART was required despite the stable level of CD4+ lymphocytes. There was the sharp decrease in the viral load after the indication of HAART (Fig. 5). There were practically no dynamics after the 1^{st} DFPP procedure in one patient (#5) who did not receive HAART, but the level of HIV RNA decreased by 92% after the third procedure (Fig. 5). This patient at the beginning and end of the study did not take HAART, as indicators of HIV viral load and the number of CD4+ did not require its use.

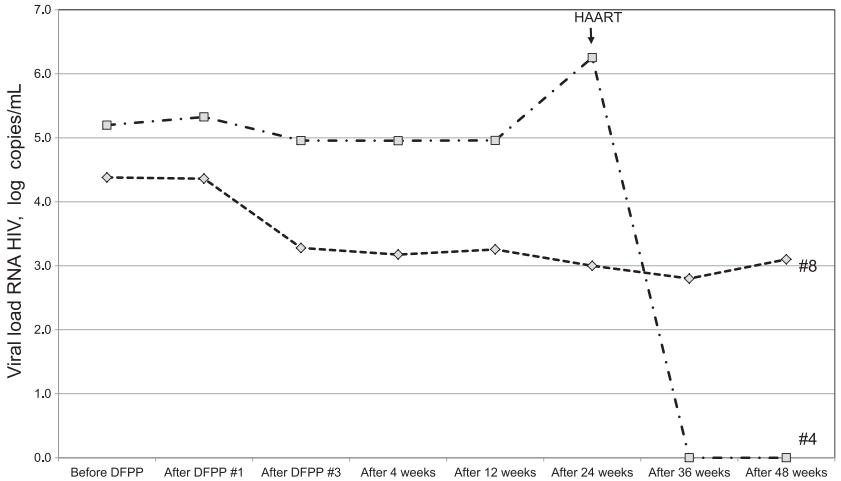

FIG. 5. Dynamics of HIV viral load during during the double filtration plasmapheresis (DFPP) + PEG-IFN -RBV in patients that were not receiving HAART.

Five of 8 patients who completed the full course of therapy had a sustained virological response (SVR) with respect to HCV. Three of them had the rapid virological response by the 4th week (RVR), two patients at the 12th week of treatment (cEVR). Two of these five patients in the past did not respond to PEG-IFN + RBV therapy, while three had a relapse after this kind of therapy. The relapse (the appearance of HCV in the blood after his disappearance) appeared in 3 of 8 patients included in the study. These patients had a history of HCV recurrence in the past. Thus, the recurrence in these patients occurred after 4, 12 and 24 weeks after the treatment.

Thus, the PEG-IFN + RBV therapy was effective in 62.5% of patients, including 50% of patients who had had a history of relapse following PEG-IFN + RBV therapy and 100%, with a history of the effect of PEG-IFN + RBV therapy was absent.

During DFPP, (treatment period and follow-up), patients had no complications or side reactions (excluding neutropenia in 1 out of 9 patients having a background of antiviral therapy), including: the development of bacterial infections, bleeding, or increased frequency of hospitalizations.

DISCUSSION

DFPP has been shown to be effective and has been used for many years in the treatment of chronic hepatitis HCV (4). However, DFPP was not previously used in the treatment of patients co-infected with HIV and HCV.

It is known that the size of HCV virus is 55–65 nm and HIV – about 100 nm, that is 2–3 times higher than the pore diameter of the fractionator Evaflux 5A20 (30 nm). During our studies the plasma volume in the patients that were undergoing the cleansing of the virus was 120% of the total volume of the circulating plasma. HCV and HIV viruses were not detected in plasma after the fractionator Evaflux 5A20, so the viruses were completely removed during the procedure. On the basis of theoretical calculations, one would expect a decrease in viral load at the end of the procedure DFPP by 60–65%. However, such a decrease was not observed in any of the eight patients. After the first DFPP procedure, the HCV viral load decreased only by 13% in one of the patients, in one patient it did not change, and in six patients it increased by 13–100%. After the third DFPP procedure the HCV viral load decreased in 4 patients by 90–99%, in two patients by 32 and 43%, in two patients it increased seven and 11 times. A similar pattern was observed for HIV (two patients had HIV in the blood). After the first DFPP the HIV viral load in one patient did not change; in the second patient it increased by 34%. After the third DFPP the HIV viral load decreased by 43 and 92%, respectively.

Such dynamics of HCV and HIV concentration in blood indicate that viruses are redistributed in the body, that they enter bloodstream from tissues and fluids. It is likely that during the DFPP treatment the entry of the virus from the tissues is reduced.

The correlation between the changes in the blood concentration of HCV and the final response to the treatment is ambiguous. The relapse of HCV infection appeared in two patients who had a decrease of viral load after three DFPP and in one patient in whom viral load after three DFPP increased. However, in four patients with the decrease in the viral load after three DFPP procedures by more than 90%, there was no recurrence.

The absolute and relative CD4+ lymphocytes count is the important indicator of HIV infection. The combined therapy that we have used did not lead to the reduction of this indicator in HIV-infected patients, which means that there was no negative impact on the course of HIV infection.

During the procedure, we observed a decrease in the total cholesterol levels by an average of 55% and a reduction in LDL-C by 73%, which is the positive factor in the treatment of HIV infection because antiretroviral therapy often leads to dyslipidemia. The decrease of the amount of cholesterol in the blood at DFPP procedure is able to neutralize the negative effect of therapy.

The presented results have confirmed the data of Japanese scientists (4) on improving of the efficiency of HCV (genotype 1) therapy by including DFPP in the treatment program. However, the data on the use of such strategy with HCV and HIV co-infection are presented for the first time.

CONCLUSION

The results of our study have shown that the positive clinical effect in the treatment of HCV patients by DFPP is also achieved in the treatment of HIV infected patients. A conclusion can be made that the complex therapy of hepatitis C, including DFPP and medication by PEG-IFN + RBV is an effective and safe approach for the treatment of HCV in patients co-infected with HCV and HIV.

New effective protocols of treatment of HIV-HCV coinfected patients have appeared to te end of the study (ledipasvir-sofosbuvir, ombitasvir-paritaprevir-ritonavir plus dasabuvir with or without ribavirin). It was initiated before these drugs appeared in the market. Furthermore, these drugs have a rather high cost. The total cost of treatment increases by $10 000–15 000, which is significantly higher than the cost of three DFPP. Therefore, the abovementioned method of treatment has the right for existence. In addition, the data obtained in the study are of value from the scientific and theoretical point of view, because we demonstrated the removal of the HIV virus in the DFPP procedure. The extracorporeal removal of viruses can be of importance in treatment of viral infections, where there is the direct correlation between the level of viremia and mortality. The first positive results of this approach in the treatment of Ebola have been published (6).

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