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Immunomodulator Gepon suppresses the replication of Hepatitis C Virus (HCV) in human cell culture *in vitro*

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The antiviral activity of the immunomodulator Gepon was studied in human cell culture, infected with Hepatitis C virus. It was found that the addition of Gepon to the cell culture, infected with Hepatitis C Virus, protected the human SW-13 cells from the cytopathogenic effect of Hepatitis C Virus. The maximum antiviral effect occurred in the presence 1 µg/ml Gepon. The control antiviral agent reaferon (interferon alpha-2a) was more a potent cytoprotecting agent in human SW-13 cells. Since Gepon changes the spectrum of cellular cytokines and interferon, it is expected that its protective action is effective against different viruses, for example Encephalomyocarditis virus.

Key words: Immunomodulator, Gepon, Hepatitis C, Reaferon.

Recently there have been communications about the successful application of the immunomodulator Gepon in virus infections, in particular, in the respiratory viral diseases [1,2] and in herpes virus infection [3,4]. It was established that the therapeutic action of agent was based not only on an increase in the effectiveness of immune reactions specific to the infectious antigens [5 to 7] but also Gepon can directly inhibit the development of virus in the infected cells. For example, Gepon impeded the development of the encephalomyocarditis virus in infected human cell culture *in vitro* [8, 9].

In this paper, the antiviral action of Gepon in human cell culture, infected with Hepatitis C Virus was studied.

Material and Methods

Virus. In this work, the cytopathogenic strain of Hepatitis C Virus (HCV) C – 13 was used, which is related to genotype 1 B [10]. The strain was isolated from the blood serum of a patient sick with chronic Hepatitis C Virus infection. It was identified as Heptatis C Virus in a neutralization test with antibodies specific to HCV, and also in a hemagglutination inhibition assay, immunoenzyme analysis, immunofluorescence and diffusion precipitation in agar. The RNA of the virus was identified as the genome HCV by RT-PCR with the use of primers to 5-NTR and to the region of HCV which codes nucleocapsid protein, and also by sequencing fragments of the HCV genome, which codes the region of nucleocapsid protein of HCV [10 —12].

Cell Culture. The cells of an adenocarcinoma form a human adrenal gland (SW-13) were obtained from the National American Cell Culture Collection. These cells were used because they are highly sensitive to the cytopathogenic action HCV. The culture of SW-13 cells was grown on 'Igla' dual medium with 10% Fetal Calf Serum, with the addition of glutamine and antibiotics (100 units/ml). A one-day monolayer of SW-13 cells grown in the 24-microwell plastic plates was used for infection by HCV. The SW-13 cells were infected with HCV at a dose of 10 TCD_{50}/ml. The infectious activity of HCV was calculated on the basis of the results of titration on 6th day after infection, when the maximum cytopathogenic action of virus had developed.

Study of the antiviral action of the agents. In this study, a sterile lyophilized preparation of Gepon-0,002g, a synthetic tetradecapeptide produced by OOO Immapharma (Moscow) was used. Preparation was dissolved in water before use, and was inoculated to final concentrations of 0.1, 1.0 and 10.0 µg/ml in the cultures of SW-13 cells at the moment of infection and 24 hours after the infection of the cells by the virus. The control preparation was reaferon (NPO "vector", Novosibirsk) at a concentration of 5000 units/ml, which has known antiviral activity against infection of the human cell culture by HCV. The antiviral action of preparations was evaluated according to decrease or elimination of the cytopathogenic action of HCV, and also by the reduction in the titer of HCV in the culture fluid of the cultures of SW-13 cells, infected with HCV at the dose of 10 TCD_{50}/ml.

Titration of HCV in the culture fluid. Samples of the culture fluid, collected on 3rd day after the infection of the cells, were used in the form of 10-fold serial dilutions for infecting a secondary SW-13 cell culture, sensitive to the replication of HCV. For this purpose 48-microwell plastic plates were used, to which a volume of 200 µl of culture supernatant under investigation was added to each well, followed by the suspension of cells in 'Igla' dual medium, containing 4% Fetal Calf Serum in a volume of 500 µl. After incubation at room temperature during 20 minutes the infected cultures were placed in the thermostat incubator for the subsequent cultivation with 37 ℃ in the atmosphere with 5% CO_2. The results of titration were studied between the 3rd and 7th day after infection, when distinct cytopathic changes in the monolayer of the cells of the infected cultures could be observed. In control uninfected cultures there was the absence of cytopathic phenomena. The formula of Reed and Mencha was used for calculating the titre HCV [13].

Results and Consideration

Protection of SW-13 cells from cytopathogenic action of HCV

The cytopathogenic action of HCV was observed in control cell cultures infected with the virus. In particular, 25% of the monolayer of the SW-13 cells was decomposed after only 3 days of infection by 10 TCD_{50}/ml of HCV.

In the preliminary experiments it was shown that Gepon in the investigated concentrations from 0.1 µg/ml to 10.0 µg/ml had no cytotoxic activity on the

SW-13 cells in the culture *in vitro*. This made it possible to use these concentrations of the agent for studying its antiviral activity.

The introduction of Gepon into cell cultures, infected with HCV, protected the SW-13 cells from the cytopathogenic action of the virus. With the aid of Gepon, it was possible to preserve the viability of 100% of the SW-13 for 3 days after their infection of 10 TCD_{50}/ml of HCV (Table 1). In this period, the control cell cultures, infected with HCV, developed the cytopathogenic phenomena, which destroyed 25% of monolayer. At the same time in the cultures which contained Gepon, the phenomena of cyto-destruction was less expressed or was absent.

In the presence 1 μg/ml of Gepon, the maximum antiviral action was achieved in which it was possible to preserve viable cell cultures for 3 days after infection by HCV (see Table 1).

The simultaneous introduction of Gepon together with HCV into the cell cultures resulted in a stronger antiviral effect than the introduction of Gepon 24 hours after the infection of cultures with HCV (see Table 1). The introduction of Gepon (1 μg/ml or 10 μg/ml) into the cell culture 24 hours after infection, increased the viability of the HCV infected SW-13 cells by 15-25% in comparison with the control infected culture of cells (3rd day after infection).

It is important to note that in spite of the antiviral action of Gepon, the cytopathogenic action of HCV nevertheless developed over longer periods after infection. On the 6th day after infection, HCV infected SW-13 cells perished both in the control cultures and in the cultures which contained Gepon.

The control antiviral preparation Reaferon possessed a more significant ability to preserve the viable properties of the SW-13 cells. In the cultures which contain reaferon at a concentration 5000 units/ml, 100% viability of cells both after 3 days and 6 days after the infection of 10 TCD_{50}/ml of HCV were observed (see Table 1).

Table 1. Influence of Gepon on the viability of SW-13 cell cultures infected with 10 TCD_{50}/ml of HCV (3rd day after infection)

Preparation	Time of addition	Viability (in %) of the SW-13*			
		Concentration of gepon in vitro, μg/ml			
		0	0.1	1	10
Gepon	At the moment of infection	75	80	100	90
Gepon	24 hours after infection	75	75	100	90
Reaferon	At the moment of infection	100	-	-	-
Reaferon	24 hours after the infection	100	-	-	-

Note: —viability of living cells (in %) on 3rd day after HCV infection and agent.

Reduction in titre of virus in cell culture

The protection of cells from cytopathogenic action of HCV is not direct proof of the antiviral properties of Gepon. The absence of the cytopathogenic effect of virus does not mean that the active production of infectious virus has stopped in the cells. The formation of chronic or persistent infection in different types of cells is a special characteristic of HCV.

In order to make a direct estimation of the antiviral action of Gepon, the HCV titre in SW-13 cell culture infected by HCV was determined. On 3rd day after infection of the cultures, the culture fluid was sampled and titrated in the secondary cultures of the cells of SW-13. Table 2 presents the results of the titration. In the control cultures on 3rd day after infection, the titre of HCV reached the value of 3,2 log TCD_{50}/ml. The presence of Gepon decreased titre of HCV in the infected cultures. 3 days after infection, the cultured fluid from cultures which contained Gepon had the minimum titre of HCV of 2 log TCD_{50}/ml (table 2).

Table 2. Influence of Gepon on the replication of HCV in the cultures of the cells SW-13, infected of 10 TCD_{50}/ml HCV

Preparation	Time of addition	Titre of HCV (log TCD_{50}/ml) in culture supernatants containing the agents indicated			
		Concentration of Gepon in vitro, µg/ml			
		0	0.1	1	10
Gepon	At the moment of infection	3.2	2.5	2.3	2.0
Gepon	24 hours after infection	3.2	3.2	3.3	3.1
Reaferon	At the moment of infection	0	-	-	-
Reaferon	24 hours after the infection	0	-	-	-

Note: - Titre of HCV (log TCD_{50}/ml) 3 days after infection of the SW-13 cell culture.

Gepon suppressed the replication HCV in the concentration range from 0,1 to 10 µg/ml, in this case the titre of HCV was reduced by 0.7-1,2 log TCD_{50}/ml. The antiviral action of Gepon was more effective when it was introduced into the cell cultures at the moment of infection, rather than after 24 hours.

The results of this investigation confirm that Gepon inhibits the replication of HCV and it effectively protects the human SW-13 cells from the cytopathogenic action of HCV (Table 1 & 2). These results agree with antiviral action of Gepon in cultures described earlier in human J-96 and L-41 cells, infected with encephalomyocarditis virus. [8, 9].

Earlier we reported that Gepon induces a significant change in the spectrum mRNA of interferons and cytokines synthesized by human J-96 cells [8]. A change in the spectrum of synthesized interferons and cytokines is the most likely basis for an increase in the stability of cells to the virus infection. The uniform changes, induced by Gepon in the different types of cells (SW-13, J-96, L-41), could be the cause of the consistent biological effects, such as inhibition of the replication of viruses and the protection of cells from the cytopathogenic action of viruses. Since Gepon changes the spectrum of cellular cytokines and interferon, its antiviral action must be effective with

respect to different viruses, for example viruses of encephalomyocarditis, herpes and hepatitis C.

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