VIROLOGY

Antiviral Activity of *Inonotus Obliquus* Fungus Extract towards Infection Caused by Hepatitis C Virus in Cell Cultures

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Fractions of *Inonotus obliquus* fungus water extract exhibited a virucidal effect towards hepatitis C virus: it 100-fold reduced its infective properties within 10 min. The antiviral effects of fungus extracts manifested after preventive (24 h before infection) and therapeutic use (during infection of porcine embryo kidney cells). Moreover, the data indicate that the birch fungus extracts inhibit production of infective virus by porcine embryo kidney cells.

Key Words: *virus; hepatitis C; cell cultures; fungus extract; fractions*

According to biological classification, fungi occupy the position between the plant and animal worlds. These organisms often contain highly active compounds providing their nutrition and defense from other biological objects. This is true primarily for long-lived fungi, such as parasitic *Inonotus obliquus* fungus (*Basidomycetes* class), living for 15-20 years in the forest atmosphere. For its parasitic mode of life, this fungus should posses a potent enzymatic system allowing wood pulp digestion and a system of defense from environmental factors, including plant viruses, spores of other fungi, various microorganisms, insect toxins, and other natural pathogens.

Water extracts from this fungus are traditionally assumed to be the sources of substances with cytostatic and cytotoxic effects and are now manufactured and sold in the form of a nontoxic aqueous extract called Befungin. The technology of collection and extraction of bioactive substances from the fungus body for the manufacture of this preparation is poorly differentiated, so that only its cytostatic activity is retained.

The antioxidant, anti-inflammatory, antiproliferative, and immunostimulatory effects of *I. obliquus* fungus have been described [4-7].

The importance of search for effective means for the treatment of hepatitis C [1] and the unique possibility to screen antiviral drugs on the model of cell culture infection by hepatitis C virus (HCV) [2,3] prompted us to evaluate the antiviral effects of water-soluble substances of the surface layers of this fungus towards HCV infection in cell cultures.

MATERIALS AND METHODS

The study was carried out on a cytopathogenic HCV variant (strain C-13) isolated from the serum of a female patient with chronic hepatitis C [2,3]. A specimen of culture fluid collected on day 6 after infection of porcine embryo kidney cell (SPEV) culture served as the virus-containing material.

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TABLE 1. Virucidal Effects of Various Fractions of *I. obliquus* Fungus after 10-min Exposure with Virus-Containing Material

Substance fraction No.	Titer of HCV infective activity	Reduction *vs.* control titration
1	Toxic	—
2	5.0±0.5	1.0
3	3.5±0.3	2.5
4	3.5±0.3	2.5
Virus control	6.0±0.5	—

Antiviral activity of extracts from birch fungus *I. obliquus* was studied on SPEV cultures sensitive to HCV reproduction. The cells were cultured for 2 days to confluence in 48-well plastic culture plates in medium 199 (M. P. Chumakov Institute of Poliomyelitis and Viral Encephalitides) with 100 U/ml penicillin and streptomycin and 10% bovine serum.

Obtaining of *I. obliquus* fractions. In order to prepare the material, a 2-3-mm layer was removed from the fungus surface, a total of 100 g; 30 g of the layer was dried at 20°C, fragmented in one liter of water, mixed with a rapid mixer for 3 days at 20°C and then 6 h more at 50°C. The suspension was then left for precipitation, after which the supernatant was collected, centrifuged at 4000 rpm, filtered through a filter with small pores, and evaporated on a rotor evaporator at 20 mm Hg (40-50°C). The resultant dry extract, dark brown glossy plates, was then fractionated by liquid chromatography in a column packed with biogel P-10. The resultant four fractions, polyphenylcarbonic acid substances of the fungal surface layer, were evaporated on a rotor evaporator and tested for antiviral activity towards HCV infection in cell cultures.

Virucidal activities of the fractions were studied after exposure of each of the four fractions with virus-containing material at ambient temperature for 10 min. Infective activity of HCV was then titered in SPEV cultures in each variant of the experiment. The fraction was assumed to possess virucidal activity if HCV titer for SPEV cells decreased at least 100-fold after the exposure.

Antiviral activity of *I. obliquus* fractions was evaluated in SPEV cultures infected with HCV. The fractions in different concentrations were added to the cultures 24 h before or simultaneously with HCV infection. Antiviral efficiency of the fractions was evaluated by their capacity to protect SPEV cells from the

TABLE 2. Antiviral Effect of *I. obliquus* Extract on the Model of HCV Infection in SPEV Cell Culture (Extracts Added 24 h before Cell Infection)

Fraction No.	Percentage of SPEV cells dead from viral infection after treatment with *I. obliquus* extract in dilutions				No extract
	1:2	1:4	1:8	1:16	
1	0	0	60	100	100
2	0	0	0	100	100
3	0	0	60	100	100
4	0	0	20	100	100

TABLE 3. Antiviral Effect of *I. obliquus* Extract on the Model of HCV Infection in SPEV Cell Culture (Extracts Added Simultaneously with Cell Infection)

Fraction dilution	Percentage of HCV infected SPEV cells dead from viral infection after treatment with different *I. obliquus* fractions				No extract
	No. 1	No. 2	No. 3	No. 4	
1:2	Toxic	Toxic	0	0	80
1:4	0	0	0	75	80
1:10	0	80	0	50	80
1:20	100	70	75	100	80

Note. Here and in Table 4: "Toxic" means the cytotoxic effect of the extract.

TABLE 4. HCV Titers in Medium Specimens Collected 48 Hours after Cell Infection (infective dose 100 TCD_{50})

Extract dilution	HCV titer in medium specimens ($lgTCD_{50}$/0.2 ml)				No extract
	No. 1	No. 2	No. 3	No. 4	
1:2	Toxic	Toxic	0	Toxic	6.0±0.5
1:4	0	0	0	3.0±0.1	6.0±0.5
1:10	0	4.2±0.3	0	0	6.0±0.5
1:20	5.3±0.5	4.5±0.3	5.1±0.3	5.2±0.3	6.0±0.5

cytopathogenic effect of the virus in comparison with the control HCV-infected cultures (without treatment).

In addition, specimens of culture fluid were collected 48 h after cell infection and treatment by the fungus fractions and the HCV infective titer was measured in these specimens, in order to evaluate the efficiency of *I. obliquus* water extracts inhibition of HCV production by infected cells.

RESULTS

Fractions of *I. obliquus* water extract exhibited virucidal activity towards HCV (Table 1). Fractions Nos. 3 and 4 exhibited the highest capacity to inactivate the virus infectivity, reducing HCV infective activity more than 100-fold. Fraction No. 4 was characterized by cytotoxic effects, which precluded evaluation of its virucidal effects.

In order to rule out the cytotoxic effects of the fungus extracts, its antiviral activity was evaluated with different concentrations of the substances.

I. obliquus extracts added 24 h before HCV infection protected SPEV cells from the pathogenic effect of HCV (Table 2).

All 4 fractions of aqueous extract of the fungus in 1:4 dilution completely protected SPEV cells from virus-induced death. Fractions Nos. 2 and 4 exhibited the highest antiviral effect under these conditions: in dilutions up to 1:8-1:16 they protected the cells from the pathogenic effect of the virus. Antiviral activity of fraction No. 1 was lower. Addition of the fungus extract fractions simultaneously with cell infection by the virus also indicated antiviral activity of the fungus substance. In these experiments, fraction No. 3 exhibited the highest antiviral effect: this fraction completely protected SPEV cells, being used in 10-fold lower dilution in comparison with other fractions (Table 3).

The production of infectious virus in infected SPEV cells after treatment by *I. obliquus* fractions was evaluated (Table 4). A significant reduction or complete absence of infective virus were found in specimens of culture medium collected 48 h after cell culture infection and treated by the fungus extract in comparison with the virus concentration in specimens of untreated cultures. Fraction No. 3 also inhibited the highest antiviral effect in this experiment. In dilutions up to 1:10 it completely suppressed the cell production of the infective virus throughout 48 h after infection, while in media of cultures not treated by the fungus extract HCV titers reached 6.0 $lgTCD_{50}$/ml.

Hence, the data attest to virucidal effects of *I. obliquus* birch fungus extracts. The fungus extracts are characterized by low cytotoxicity and high antiviral effects, protecting 100% SPEV cells from virus-induced death after cell infection in a dose of 0.1 TCD_{50}/cell. The antiviral effects of the fungus extracts manifested after their preventive use 24 h before infection and therapeutic use simultaneously with cell infection. These data also indicate that the birch fungus extracts suppress the production of infective virus by the cells.

Hence, the need in further studies of antiviral activity of *I. obliquus* fungus towards the infection caused by HCV is obvious.

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