Antiviral Activity of Trichothecene Mycotoxins (Deoxynivalenol, Fusarenon-X, and Nivalenol) against Herpes Simplex Virus Types 1 and 2

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Abstract: The effect of trichothecene mycotoxins, deoxynivalenol (DON), fusarenon-X (FX) and nivalenol (NIV), on plaque formation of herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) in HEp-2 cells was examined. The 50% effective concentrations (EC50) of DON, FX, and NIV for HSV-1 plaque formation were 160, 56, and 120 ng/ml, respectively. Those for HSV-2 plaque formation were 94, 26, and 50 ng/ml, respectively. These three mycotoxins showed about 2-fold higher selectivity to HSV-2 than to HSV-1. Plaque formation of HSV-1 was not inhibited with trichothecenes at concentrations completely inhibiting plaque formation when cells were treated during virus adsorption period or 15 hr before infection. These results indicate that trichothecenes affect replication of HSV-1 after virus adsorption, but not before or during virus adsorption to the host cells.

Key words: Antiviral activity, Trichothecene, Herpes simplex virus type 1 and type 2

Trichothecene mycotoxins such as deoxynivalenol (DON), fusarenon-X (FX), and nivalenol (NIV) produced by Fusarium species of fungi are a family of sesquiterpenoid compounds and have a double bond at C-9 and C-10 and an epoxy ring at C-12 and C-13 of the molecules (Fig. 1). They belong to group B of trichothecenes, having a carbonyl group at C-8 of the molecules, and are characterized by the presence of either a hydrogen atom or a hydroxyl group (or an acetyl group) at C-4 of the molecules without a macrocyclic ring between C-4 and C-15 (12). Although these trichothecenes have various biological properties (1, 3, 5, 10, 11), little study on their antiviral activities against animal viruses has been reported.

In this study, we evaluated the antiviral activities of trichothecenes against herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) by the plaque reduction test. In addition, the effects of the toxins on HSV-1 plaque formation were examined both by treatment during the virus adsorption period and by treatment before virus infection.

HSV-1 strain HF and HSV-2 strain UW-268 were used in this study. Human epidermoid carcinoma No.2 (HEp-2) cells used as host cells were grown in Eagle’s minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS). NIV was purified from moldy barley infected with Fusarium graminearum (F-1465) by centrifugal partition chromatography as described previously (8, 9), and DON and FX were purchased from Wako Pure Chemical Industries, Ltd., Japan. These compounds were dissolved in dimethyl sulfoxide at a concentration of 20 mg/ml and diluted in Eagle’s MEM. Stock solutions (200 μg/ml) were prepared, passed through a 450-nm membrane filter (Millipore Corp., U.S.A.), and stored at −20°C until use.

The cytotoxicity of the three toxins for HEp-2 cells was monitored by inhibition of cell growth. HEp-2 cells were seeded at 5 × 10⁴ cells/well in 24-well tissue culture plates and allowed to proliferate at 37°C for 24 hr in growth medium. The medium was then replaced with new medium containing different concentrations of the test compounds. After an additional 3 days of incubation at 37°C in 5% CO₂, the number of viable cells was counted by trypan blue exclusion after trypsinization. The cytotoxicity of the toxins is expressed as the 50% inhibitory concentration (IC50), which is the concentration required to reduce cell growth by 50% of the control.

Abbreviations: DAS, diacetoxyscirpenol; DON, deoxynivalenol; EC50, 50% effective concentration; FBS, fetal bovine serum; FX, fusarenon-X; HEp-2, human epidermoid carcinoma No.2; HSV, herpes simplex virus; IC50, 50% inhibitory concentration; MEM, minimum essential medium; NIV, nivalenol; T-2, T-2 toxin.
The IC$_{50}$ of DON, FX, and NIV were 1,420, 488, and 944 ng/ml, respectively (Table 1).

Confluent monolayers of HEp-2 cells in 60-mm plastic dishes (two dishes/dose) were infected with 200 plaque forming units of HSV-1 or HSV-2. After 1 hr of virus adsorption, the cultures were overlaid with 1.5% methylcellulose in Eagle's MEM containing 5% FBS with or without trichothecenes and then incubated at 37°C. The plaques were visualized with 0.02% neutral red at 2 days after infection and counted on the following day. Plaque formation of HSV-1 and HSV-2 was inhibited by the compounds in a dose-dependent manner, and the effective concentrations required for 50% inhibition of plaque formation (EC$_{50}$) of DON, FX, and NIV for HSV-1 were 160, 56, and 120 ng/ml, respectively. Those for HSV-2 plaque formation were 94, 26, and 50 ng/ml, respectively (Table 1). This result showed that the antiviral activity of FX was greater than DON and NIV, suggesting that the presence of an acetyl group for FX at C-4 is important for the antiviral activity. The selectivity indexes (the IC$_{50}$/EC$_{50}$ ratio) of each compound were calculated. The values of DON, FX, and NIV for HSV-1 were 8.9, 8.7, and 7.9, respectively, and the values for HSV-2 were 15.1, 18.8, and 18.9, respectively.

Okazaki et al (6, 7) reported that the EC$_{50}$ of group A of trichothecenes such as diacetoxyscirpenol (DAS) and T-2 toxin (T-2) against HSV-2 were 2.6 and 2.3 ng/ml, respectively, and the values of the macrocyclic trichothecenes such as baccharinoid B-5, roridin A, and satratoxin G were 8.6, 1.4, and 1.5 ng/ml, respectively. These findings suggest that the antiviral activities of group B of trichothecenes (DON, FX, and NIV) against HSV-2, probably, also against HSV-1 were lower than those of group A and macrocyclic trichothecenes.

The effects of trichothecenes on plaque formation of HSV-1 were examined when cells were treated during the virus adsorption period or the host cells were treated for 15 hr before virus infection (Table 2). The treatment of the host cells with the toxins for the adsorption period (1 hr postinfection) did not affect plaque formation of HSV-1. In addition, plaque formation of HSV-1 was not
inhibited by pretreatment of HEp-2 cells with the toxins for 15 hr before HSV-1 infection, suggesting that the cell membrane changes, which might prevent adsorption and penetration of HSV-1 virions into the host cells, were not caused by pretreatment of the cells with trichothecenes.

It has been reported that trichothecenes inhibit the initiation or elongation/termination step in protein synthesis of eukaryotic cells by binding to the 60S subunit of ribosomes at the peptidyl transferase site (2, 4). Therefore, the inhibitory effects of DON, FX and NIV, on HSV-1 plaque formation can be brought about by binding of the compounds to the polyribosomes in the viral protein syntheses.

References


