Inhibition of rubella virus growth by Fungizone

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Received 21 June 2000; received in revised form 15 September 2000; accepted 26 September 2000

Abstract

Fungizone added to agar overlay medium inhibited plaque formation in both size and number by rubella virus in rabbit kidney 13 cells. In the presence of 1 µg/ml of Fungizone, the diameter of the plaques was reduced to one half of that in the absence of the drug, and at 5 µg/ml, plaque formation was inhibited by 80%. When the drug was added to the culture medium, the growth of infectious virus was also inhibited with reduction in the synthesis of envelope glycoprotein E1 and capsid protein C in infected cells. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Rubella virus; Fungizone; Antiviral effect

Rubella virus, a member of Togaviridae, is an enveloped positive-stranded RNA virus. The viral particle possesses glycoproteins E1 and E2 in the envelope and capsid protein C; their molecular masses (Mr) have been reported to be 57, 42–47, and 33 kDa, respectively [1,2]. Rubella is an exanthematous systemic virus infection among both children and adults. The most serious problem of rubella virus infection is congenital rubella syndrome (CRS), caused by infection [3].

Rubella as well as CRS has been controlled by vaccination in USA [4]. However, in countries where the vaccination is incompletely carried out, rubella epidemics and CRS cases still occur even now [5].

Under these situations, the necessity and importance of rubella diagnosis by virus isolation have not been lessened. Moreover, it is necessary to distinguish the isolates from vaccine strain and to identify the virus origin.

Virus isolation and titration in tissue culture cells require long-term incubation. During the incubating period, addition of Fungizone into the overlay medium is recommended to avoid mycotic contamination [6]. We have found, however, that addition of Fungizone to the agar overlay medium would reduce the plaque formation of rubella virus. Therefore, we tested the effects on the growth of rubella virus of this polyene antibiotic, which is widely used for tissue culture systems as well as in clinical medicine.

To-336 strain, a rubella vaccine strain (Takeda Chemical Industries, Inc., Osaka) and M-33 strain, a wild virus strain, grown in BHK21 cells were used in the present study. RK 13 cells, a rabbit kidney cell line, were used for virus growth and infectivity assays. Fungizone, composed of amphotericin B and sodium deoxycholate (DOC), an emulsifying agent, was purchased from Bristol-Myers Squibb K.K. (Tokyo). Mouse monoclonal antibody (MAb) 85 [7] and MAb 116 (Y. Umino, unpublished data) to rubella virus E1 and C proteins, respectively, were purified as an IgG fraction. Swine anti-rubella antiserum was purified as an IgG fraction, having a titer of 27 HIU/µg. To obtain anti-E2 peptide serum, a 12-amino acid peptide (PELVSPMGRATC) corresponding to the amino acid residues 208–219 of rubella virus E2 was synthesized (Fujiya Bio-Science Institute, Kanagawa), conjugated to keyhole limpet hemocyanin and injected subcutaneously into a rabbit with Freund’s complete adjuvant. The infectivity of rubella virus was titrated by the plaque assay method in RK13 cells as described previously [8]. Statistical significance of the difference of plaque size and number between control and the presence of drug was assessed by Student’s t-test. For
radioisotope labeling of virus and infected cells, RK13 monolayers infected with M-33 strain at a multiplicity of infection (MOI) of 3 PFU/cell were added with 3 ml of EMEM containing 2% CS with various concentrations of Fungizone and 2.96 MBq [35S] methionine for 8 h. The labeled cells and the virus collected from culture supernatant were each dissolved in radioimmunoprecipitation (RIP) buffer [9]. The methods for RIP, one-dimensional SDS-PAGE, two-dimensional isoelectric focusing by use of Pharmalyte (pH 3–10) and fluorography were done as described [7,10].

To observe the effects of Fungizone on plaque formation, RK13 cells, infected with To-336 and M-33, were overlaid with medium containing 1–20 µg/ml of the drug and incubated at 35°C. The diameter of the plaques of To-336 strain and the ratios of the plaque number of To-336 and M-33 strains formed in the presence of Fungizone to those in the absence of the drug are shown in Fig. 1. In the presence of 1 µg/ml of Fungizone in the agar overlay medium, the diameter of the plaques formed by To-336 strain was reduced to 50% (P < 0.01) and their number to 70% (P < 0.1). In the presence of 5 µg/ml of the drug, the plaque size was further reduced to 40% (P < 0.01), and the number to 20% (P < 0.01) of control. The plaque formation by M-33 strain was similarly inhibited as that by To-336 strain. The addition of DOC alone at 20 µg/ml, which is present in the Fungizone solution as an emulsifying agent, inhibited plaque formation by To-336 strain at utmost by 20%, but no inhibition was noted at lower concentrations (data not shown).

The presence of Fungizone or DOC with To-336 virus during adsorption period for 1 h hardly affected the number of plaques, indicating that these drugs had negligible inhibitory effect on the virus adsorption and entry processes (data not shown).

As shown in Fig. 2, in RK13 cells infected with M-33 and To-336 strains, the virus yield was decreased by Fungizone in a dose dependent manner. In the presence of 20 µg/ml of the drug, the virus yield of both strains was decreased to as low as 1/1000 of those in the absence of the drug. The effect of the time of Fungizone addition to the infected cells on one-step virus growth was examined...
Fig. 4. Synthesis of intracellular virus proteins and formation of virus particles in the presence of Fungizone and analysis by two-dimensional electrophoreses. [³⁵S] methionine-labeled virus proteins in the cells (A) and in the virions (B) were immunoprecipitated using anti-rubella virus serum (a), anti-E1 MAb (b), anti-E2 peptide antibody (c), anti-C MAb (d) and analyzed by SDS-PAGE under reducing condition. 1, no Fungizone; 2, 10 μg/ml; 3, 20 μg/ml of Fungizone, respectively. Immunoprecipitates with anti-rubella virus antibody, A (a) 1 and 3, were analyzed by two-dimensional isoelectric focusing and SDS-PAGE under reducing condition. C, no Fungizone; D, 20 μg/ml of Fungizone.

(Fig. 3). The presence of the drug from 6 to 24 h after infection inhibited the virus growth in a similar fashion to the drug effect throughout the incubation period. However, the presence of the drug for 6 h immediately after virus adsorption did not inhibit the virus growth at all. The viability of uninfected cells was kept in the presence of the drug throughout the incubation period irrespective of the drug concentration.

The amounts of viral proteins E1, E2 and C synthesized in infected cells were decreased depending on the concentration of Fungizone (Fig. 4A). Since the ratio among the amount of the proteins was maintained, synthesis of each viral protein could be suppressed by a common mechanism. Inhibition of synthesis of the viral proteins was consistent with the reduced production of infectious progeny virus (Fig. 2). Microheterogeneity found in E1 and E2 proteins seemed to become narrowed. The amount of viral proteins E1, E2 and C incorporated into the progeny virus was apparently reduced at the drug concentration of 10 μg/ml or higher (Fig. 4B).

To examine a possible inhibition of the processing of oligosaccharide attached to the glycoproteins, the immunoprecipitates with anti-rubella antibody described above were further analyzed by two-dimensional electrophoreses (Fig. 4C, Fig. 4D). In the presence of 20 μg/ml of the drug, the broad band of E1 became narrow and the isoelectric points of the E1 and E2 bands were shifted slightly toward the alkaline side, resulting in distribution of wider range of isoelectric points (Fig. 4D). No change of the isoelectric point of C was observed irrespective of the presence of the drug. These results suggested that Fungizone also impaired processing of oligosaccharide side chains of the glycoproteins.
Fungizone was shown to inhibit both plaque formation and the production of infectious rubella virus. When DOC was used alone, no inhibition was observed on plaque formation or on growth. Therefore, the antiviral effect of Fungizone on rubella virus was attributable to amphotericin B, the main component of Fungizone.

It is reported that amphotericin B binds specifically to ergosterol in the cell membrane of fungi and cholesterol in the membrane of mammalian cells, and forms a water-soluble channel in the membrane, enhancing its permeability [11].

The marked inhibition by the drug at a low concentration added to virus infected cells after virus adsorption period suggested that Fungizone did not directly affect the virus. The drug exposed to the infected cells before 6 h post infection did not reduce the virus yield, indicating that the drug affected in a late stage of virus replication cycle. Even at the highest drug concentration tested, no significant change occurred in the viability of cells. This could exclude a possibility that the antiviral effect of the drug was induced directly due to cytotoxicity.

Fungizone had no effects on either plaque formation or virus growth by measles and mumps viruses in Vero cells (data not shown). Host cell proteins required for replication of measles virus [12] may not be affected by Fungizone, while those for rubella virus replication may be impaired by the drug. Differing from antiviral effect of amphotericin B reported for hepatitis B virus [13] and human immunodeficiency virus [14], for which the drug interacts with the viral envelope and at the early stage of virus infection, respectively, Fungizone acted in the late stage in the case of rubella virus. During post translational processing, E1 and E2 receive modifications such as addition and processing of oligosaccharides and fatty acids [15]. It remains unclear whether the change in the isoelectric point of intracellular E1 was attributed to inhibition of oligosaccharide modification by the drug (Fig. 4).

The present study showed that Fungizone suppressed rubella virus growth by affecting the virus protein-synthesizing process in tissue culture cells. This inhibitory effect of Fungizone should reduce the efficiency of virus isolation and plaque formation. As a result, when Fungizone is added, virus infectivity would be estimated at lower titers. It is therefore advisable not to use Fungizone at least in the potency test of rubella virus vaccines.

Acknowledgements

We thank the late Dr A. Sugiura for his valuable advice and encouragement and Dr T. Kohama for his advice and critical reading of the manuscript. The present work was supported in part by a Grant-in-Aid for Health Science from the Ministry of Health and Welfare.

References