Studies on Transmissible Gastroenteritis of Swine III. The Effect of Selective Inhibitors of Viral Replication on a Cytopathogenic Virus from Transmissible Gastroenteritis

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SUMMARY

The effect on the plaque production of the Purdue strain of cytopathogenic virus from transmissible gastroenteritis of swine by 5-bromo-2'-deoxyuridine (BUDR), 5-iodo-2'-deoxyuridine (IUDR), actinomycin-D, puromycin, and amantadine-HCI (Symmetral) has been studied.

Amantadine-HCI reduced the plaque-forming units of virus per ml by approximately 98%. Puromycin prevented almost all virus reproduction while actinomycin-D caused approximately a 22% reduction. Both IUDR and BUDR produced approximately a 20% increase in plaque-forming units of virus per ml.

Swine testis cells stained with acridine orange early in the course of infection contained brick-red particles in the cytoplasm, indicative of a ribonucleic acid (RNA) type virus.

Introduction

The successful passage in cell culture of a cytopathogenic virus from swine tissues infected with transmissible gastroenteritis (TGE) has been reported (1, 2, 3, 4). Cell culture fluids containing cytopathogenic virus produced signs of TGE in susceptible swine. Swine that recovered were resistant to TGE when exposed to TGE-infected tissue, and antiviral activity was found in convalescent serum from both experimentally and naturally infected swine (2, 4).

Selected characteristics of a cytopathogenic virus common to five isolates from TGE indicated that the virus might be a myxovirus (5). The results of the study reported here, using selective inhibitors of viral replication and acridine orange staining of infected and non-infected cultures, gave further evidence that this virus is a ribonucleic acid (RNA) type virus.

Materials and Methods

Virus Isolate and Cell Culture

The Purdue strain of virus was used as inoculum throughout these studies and was purified by reisolation from a single infected plaque in a monolayer of an established culture of swine testis (ST) cells (5). The virus was subsequently propagated in this same cell culture system

Inhibitors

Two halogenated pyrimidine nucleosides,[1] 5-bromo-2'-deoxyuridine (BUDR), and 5-iodo-2'-deoxyuridine (IUDR), actinomycin-D,[2] puromycin,[1] and amantadine HCI[3] were used.

IUDR, BUDR, amantadine-HCI (50 μg per ml of culture fluid), and actinomycin-D (0.02 μg per ml of culture fluid) were add-

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[2]Merck, Sharp & Dohme, Research Laboratories, Rahway, N.J.
[3]Symmetral, E. I. du Pont de Nemours & Co., Stine Laboratories, Newark, Del.

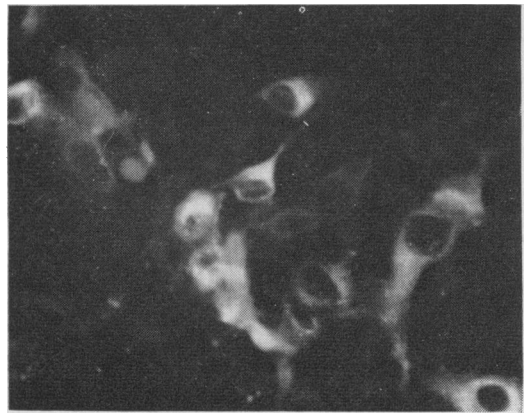

Fig. 1. Virus infected ST cells in the absence of IUDR, stained with fluorescent antibody.

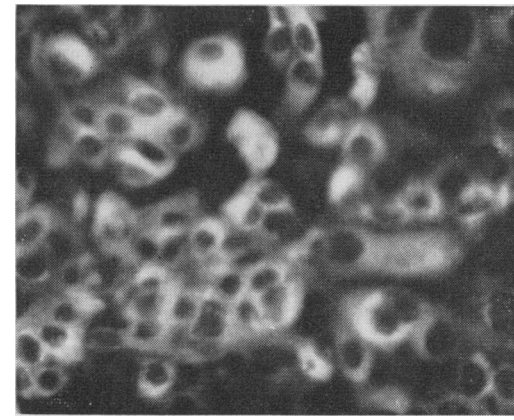

Fig. 2. Virus infected ST cells in the presence of IUDR, stained with fluorescent antibody.

ed to the cell cultures 18 hours prior to inoculation of virus. Puromycin (2 μg per ml of culture) was added immediately after inoculation of virus. At the time of inoculation, the culture fluid was removed and 2 ml of virus, containing approximately 7.9 x 10^7 plaque-forming units (p.f.u.) of virus per ml, was added and allowed to adsorb for 10 minutes. The inoculum was decanted and the cell sheet washed twice with inhibitor free medium. Fresh medium containing the appropriate inhibitor was added and the cultures were incubated at 37°C for 24 hours.

To assess the effect of the inhibitors on viral replication, the cultures were frozen and thawed once, then treated with ultrasonic vibration at 10 KC for 30 seconds in a Raytheon sonic oscillator Model DF 101. Tissue debris was removed by low-speed centrifugation and the supernatant fluids were stored at -85°C until plaque counts could be made.

Determining the Effect of the Viral Inhibitors on Replication of the Virus

A preliminary titration of the supernatant fluids from IUDR, BUDR, actinomycin-D treated cultures indicated that a 10^{-6} dilution of the supernatant fluids produced a number of isolated plaques which could be counted. For amantadine-HCI and puromycin treated cultures, dilutions of 10^{-4} and 10^{-2}, respectively, were found to be optimal.

Ten 60-mm plastic plates, 0.1 ml inoculum per plate, were used to determine the plaque-forming units per ml of virus. After 10 minutes' adsorption, the inoculum was removed and agar overlay was added. The plates were incubated at 37°C in 5% CO_2 atmosphere for 48 hours, after which 5 ml of 0.01% neutral red in 1% Noble agar was added to each plate. The plaques were counted after an additional 12 to 18 hours' incubation.

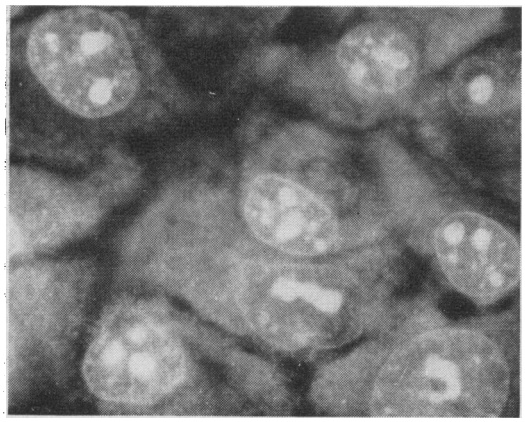

Fig. 3. Normal ST cells stained with acridine orange.

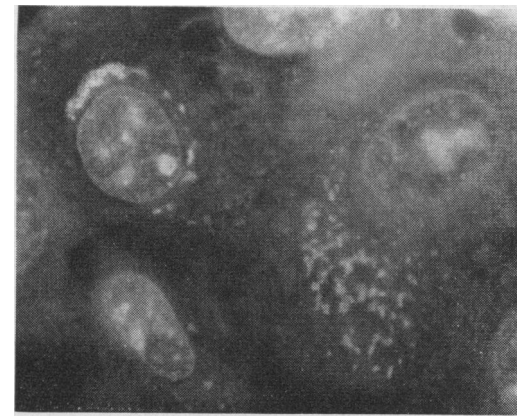

Fig. 4. Virus infected ST cells stained with acridine orange after 8 hours' incubation.

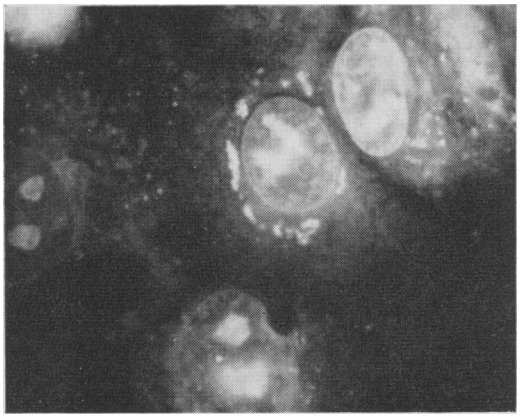

Fig. 5. Virus infected ST cells stained with acridine orange after 10 hours' incubation.

The effect of an inhibitor on the replication of virus was determined by comparing the number of p.f.u. of virus per ml from cultures treated with inhibitor with the results obtained from untreated cultures.

The Effect of IUDR and BUDR on Immunofluorescence and Cell Staining with Acridine Orange

Cover slip preparations of ST cells were treated with IUDR and BUDR 18 hours prior to inoculation with virus. Virus preparations on inhibitor treated cells and nontreated cells were fixed 8 to 12 hours after inoculation and stained with specific fluorescein-conjugated globulin or with acridine orange as previously described (5).

Results

The Effect of Selected Inhibitors on Virus Replication

The average plaque-forming units of virus per ml from 3 different passages of virus are shown in Table I. It was evident that both BUDR and IUDR allowed approximately a 20% increase in p.f.u. of virus per ml, whereas actinomycin-D decreased the p.f.u. of virus per ml by 22%. Amantadine-HCl reduced the p.f.u. per ml by 98.5% and puromycin reduced the p.f.u. per ml by more than 99%.

The Effect of BUDR and IUDR on Immunofluorescence and Cell Staining with Acridine Orange

When drug treated virus-infected cells were stained with fluorescein-conjugated antibody 8 to 12 hours after inoculation, they showed an increase incidence of cell staining and a greater intensity of cell-antigen fluorescence than with nontreated virus-infected cells. (Compare Figures 1 and 2.)

Many infected cells stained with acridine orange 8 to 10 hours after inoculation contained brick-red staining material in the cytoplasm. (Compare Figures 3, 4, and 5.) The nuclear changes previously described (5) did not generally appear until after 16 to 20 hours of incubation when most of the cells were showing degenerative changes. The incidence and intensity of the brick-red staining material were not visibly altered by the presence of BUDR, IUDR, or actinomycin-D.

Discussion

The replacement of DNA thymidine of mammalian viruses with halogenated pyrimidine nucleosides inhibits the production of complete infective virus (6, 7). Proper treatment of the cell culture which propagates a DNA virus with the selected halogenated nucleoside will result in a decrease of the infectivity, indicating a DNA type virus.

The titer of the virus reported in this paper actually increased, as measured by plaque-forming units, indicating that the virus is a RNA type virus. The reasons for the increased infectivity titer and the improved staining with fluorescein conjugated antibody following addition of IUDR or BUDR to the cell culture medium are not apparent.

Actinomycin-D has been shown to reduce the infectivity titer of influenza virus (8) and DNA dependent RNA viruses (9). The reduction of p.f.u. of this virus following the addition of actinomycin-D into the cell culture medium indicates a DNA dependent

TABLE I. Average plaque-forming units of virus per ml from 3 different passages of virus produced in the presence of selective inhibitors and in the absence of inhibitors

No inhibitor	BUDR 50 μg/ml	IUDR 50 μg/ml	Actinomycin-D 0.02 μg/ml	Amantidine-HCl 50 μg/ml	Puromycin 5 μg/ml
7.9×10^7	1.2×10^8	1×10^8	6.2×10^7	1.3×10^6	5×10^4

RNA virus of the influenza type.

Amantadine-HCI has been shown to interfere with the adsorption of influenza and parainfluenza viruses onto the host cell, thus preventing subsequent invasion and replication of infective virus (10). The marked plaque reduction which occurs following the addition of amantadine-HC to the medium of cell cultures on which this virus is growing indicates that the virus has some of the characteristics of the influenza and parainfluenza viruses.

Puromycin interrupts polio virus maturation by an apparently direct inhibitory effect on the synthesis of virus-directed protein (11). The mechanism of inhibition of replication by puromycin was not determined for the virus reported in this study.

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