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Original Article

Poliovirus Particles do not Form in Preinfected Cells with Reovirus

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Abstract

Background and Aims: Inhibition of viral growth in coinfected cells with two different viruses has been described. This phenomenon known as viral interference can occur in several virus host systems such as interference of enterovirus infection on poliovirus vaccine strains. In this study we superinfected reovirus infected HeLa cells with poliovirus to determine if poliovirus can replicate in such cells and form mature virus particles.

Methods: Cells were infected first with reovirus then were reinfected with poliovirus. The amounts of viral particles were measured by electron microscopy and plaque assay titration. The amount of viral yield was also measured using the technique of real time RT-PCR for measuring the viral load in infected cells.

Results: In cells infected first with reovirus and then superinfected with polio virus, only reovirus particles were produced. Virus production was determined by assaying viral titer using the plaque assay technique and electron microscopy. There was no poliovirus particles observable in the superinfected cells. The amount of poliovirus load in reovirus infected cells was also drastically reduced.

Conclusion: The growth of poliovirus was inhibited in reovirus infected cells and no infectious poliovirus particles could be observed. This observation could be important to consider in poliovirus vaccination program.

Keywords: Poliovirus; Reovirus; Interference; Coinfection

Introduction

The phenomenon of viral interference or the inhibition of virus growth by another virus has been known for several viruses and the mechanism of such phenomenon has been partly described (1, 2). In this phenomenon, infection by a first virus results in resistance of cells or tissues to infection by a second virus (3).

Interference has been described in certain systems such as: serial passages of viruses at high moi, resulting in accumulation of defective interfering viruses (4, 5), mixed infection of wild type viruses with certain temperature-sensitive mutants (6, 7), coinfection of cells with different wild type virus isolates (8, 9) and coinfection of cells with different genus of viruses belonging to the same or different families. An example of such interference is the effect of enterovirus infection with live - attenuated poliovirus vaccine resulting in inefficiency

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of vaccine - induced protection (10, 11). Also, combination of hepatitis A and B vaccine has been shown that the hepatitis B portion of the vaccine did not produce clinically acceptable antibody due to immunologic interference (12, 13). In another report, in viral hepatitis, acute hepatitis C virus superinfection on HBV chronic carriers caused an inhibition of the HBV genome replication (14). Greer et al (15) observed that rhinovirus infection may render the host less likely to be infected with other respiratory viruses.

In animals this phenomenon was also studied. In rainbow trout (Oncorhynchus mykiss) infected with Infectious Pancreatic Necrosis Virus (IPNV), superinfection with Infectious Haematopoietic Necrosis Virus (IHNV) inhibits the growth of the latter (16).

The other example is the effect of reovirus on environmental detection of enteroviruses (17).

In this study we have examined coinfection of cells with reovirus and poliovirus. These viruses can coexist in alimentary tract and their interference might have some impact on the outcome of their infection or may result in inefficiency of live-attenuated poliovirus vaccine. In this study we report the results of experiments obtained from intracellular coinfection of cells with reovirus and poliovirus.

Methods

Cells and viruses

For propagation of reovirus murine L cell line was used. The cells were grown in monolayers using Joklick′s Minimal Essential Medium supplemented with 5% fetal bovine serum (Gibco). For propagation of poliovirus vero cells were used. These cells were grown in DMEM containing 5% fetal bovine serum. The cells were grown at 37°C in an atmosphere containing 5% CO_2. Reovirus type 3 was propagated in L cells and used as stock for virus purification. Poliovirus vaccine strain obtained from Razi Vaccine and Serum Institute was propagated in vero cells and titrated by plaque assay method.

Superinfection

Vero cells were infected with reovirus and after 2 hours incubation at 37°C the inoculum was removed and the cells were superinfected with poliovirus (moi of 20). The infected cells were incubated in DMEM at 37°C in an atmosphere of 5% CO_2.After 32 hours the infected cells were harvested and the virus yield was assayed by $TCID_{50}$ and plaque assay method.

In order to block the growth of each of the viruses in mixed infection specific neutralizing antibody was used in the assay system. Therefore to assay the yield of poliovirus, anti reovirus was used to neutralize reovirus and for reovirus assay polio antiserum was applied.

Electron Microscopy

Coinfected cells were harvested at 24 hrs post infection. The cells were centrifuged at low speed for 15 minutes. The pellet was resuspended in 100 μlit of O.1M Tris buffer PH 7.5. The infected cells were freeze-Thawed 5 times to release the intracellular virus particles. The tubes were centrifuged in a microfuge at 8000 rpm for 3 minutes. The supernatant was carefully removed and used for negative staining. A small sample (10 μlit) was placed on a formvar coated grid and negatively stained with phosphotungstic acid (PTA).

For observation of intracellular virus, monolayers of superinfected cells were prepared as above. At 24 hrs post infection, they were fixed in 2.5% glutaraldehyde for 3 hrs. After washing in O.1M Phosphate buffer they were postfixed in 1% osmium tetroxide for 3 hrs. The fixed cells were embedded in Araldite and sectioned by a diamond knife.

Sections were stained with uranyl acetate followed by lead citrate and examined in a Zeiss electron microscope.

Determination of viral load

Monolayer of approximately 10^5 cells were infected with reovirus at moi of 20 and after 2 hrs they were super infected with poliovirus at the same moi. The cells were harvested after 24 hrs and the cell pellet was suspended in l ml

of PBS, and 100μlit were taken and RNA was extracted according to the manufactures instruction. Two μlit of the extracted RNA was used for real time RT- PCR.

Oligonucleotide primers and probe were designed as follow; Forward primers,
5′-CCCTGAATGCGGCTAATCC-3′
and reverse primers,
5′-ATTGTCACCATAAGCAGCCA-3′.
The probe was
FAM 5′-ACCGACTACTACTTTGGGTGTCC GTGTTTC-3′ BHQ1. The primers were designed from 5'NTR which is conserved and is specific for all enterviruses. So using these primers will detect enteroviruses including poliovirus. Real-time RT-PCR was performed in instruction accordance with the manufacturer′s (Life Technologies) instructions in a 9600 DNA Thermocycler. Polymerase chain reaction was performed with an initial cycle of 94°C for 10 minutes followed by 45 cycles at 94°C for 20S, 60°C for 40S,72°C for 40S. Sample were done triple test. A negative control was processed after every fourth test tube.

Results

Virus yield in coinfected cells

Monolayer of vero cells were infected with reovirus and poliovirus at different times and conditions as described in materials and methods. Control infected cells were infected with each of the two viruses at the same moi and were incubated similarly. After 32 hours cultures were harvested and the amount of virus yield was determined. In order to assay poliovirus, the harvested virus from mixed infected cells was first incubated with specific antisera prepared against reovirus. Similarly antisera against poliovirus were used for assaying reovirus. The amount of antisera used was first tested to completely neutralize the specific virus in the assay system. Therefore, the CPE observed in $TCID_{50}$ assay in presence of poliovirus antiserum was related to reovirus. As it is shown in Table. 1, the cells infected with reovirus for 2 hours followed by reinfection with poliovirus after the first passage there was a marked decrease in poliovirus production of about 7 logs as assayed by $TCID_{50}$ method.

Similar results were obtained by plaque assay for poliovirus yield. When the cells were infected with poliovirus followed by reinfection with reovirus, poliovirus multiplication was not affected whereas the reovirus yield was decreased. The decline in poliovirus replication was similar when the reovirus infected cells at 4 hours were superinfected with poliovirus.

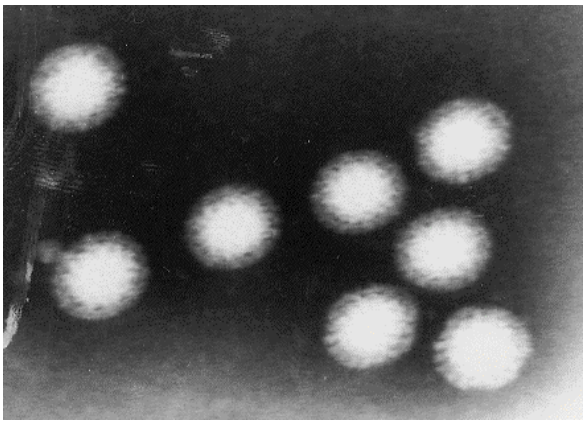

Fig. 1. Reovirus particles in extract from the poliovirus superinfected cells. There is no detectable poliovirus particles (×100000).

Electron Microscopy

In order to determine morphologically which one of the two viruses were produced in poliovirus superinfected cells, the infected cells were harvested 24hrs after infection and the cell extracts were examined by negative staining electron microscopy. As it is shown in Fig.1 only reovirus could be observed and no poliovirus particles were present indicating that mature poliovirus was not formed in reovirus infected cells. These observations were confirmed when the sections of infected cells were examined by electron microscope. It was found that reovirus infected cells when superinfected by poliovirus at 2 hrs post infection, they contained only intracytoplasmic reovirus particle (Fig. 2a).

In control poliovirus extract of infected cells, poliovirus particles were observed in the preparation by negative staining (Fig. 2b).

The results suggested that formation of complete viral particles in reovirus infected cells was inhibited.

Table 1. Poliovirus and reovirus yield in coinfected cells.

Experiment \ Passage number	1	2	3
Control poliovirus	$5\times10^{7.7}$	$5\times10^{7.1}$	5×10^{8}
Control reovirus	$5\times10^{6.1}$	$5\times10^{6.7}$	$5\times10^{6.7}$
Poliovirus yield in cells were infected first with reovirus and 2hrs later with poliovirus	$5\times10^{1.2}$	0	0
Reovirus yield in cells were infected with poliovirus and 2hrs later superinfected with reovirus	$5\times10^{5.7}$	$5\times10^{6.2}$	$5\times10^{6.5}$

Determination of viral load

The approximate number of poliovirus particles formed inside the superinfected cells was determined indirectly by viral load assay. The results showed that in control poliovirus infected cells the amount of viral load was approximately 900000/ml whereas in superinfected cells viral load was significantly reduced to the negligible level (Table. 2). The results indicated that poliovirus growth was inhibited in reovirus infected cells.

Discussion

Interference between viruses belonging to the same family or between members of unrelated ones upon coinfection of cells has been reported (18, 19).

Generally, Infection of a cell with two viruses could result in growth and maturation of both viruses, which might be beneficial to one of the viruses such as coinfection by adenovirus, and adeno-associated viruses. Coinfection could also end up in growth inhibition of one of the viruses by the other such as infection of cells With enteroviruses, which would cause growth inhibition of poliovirus (10, 11). This kind of interference has significant application value in certain systems. An example of such interference is the effect of enterovirus infection with live - attenuated poliovirus vaccine which slows down replication of vaccine virus inside the cells, resulting inefficiency of vaccine - induced protection (10, 11). The other example is the effect of reovirus on environmental detection of enteroviruses (17).

In this study we wanted to test the interference between reovirus and poliovirus, which can coexist in alimentary tract. We

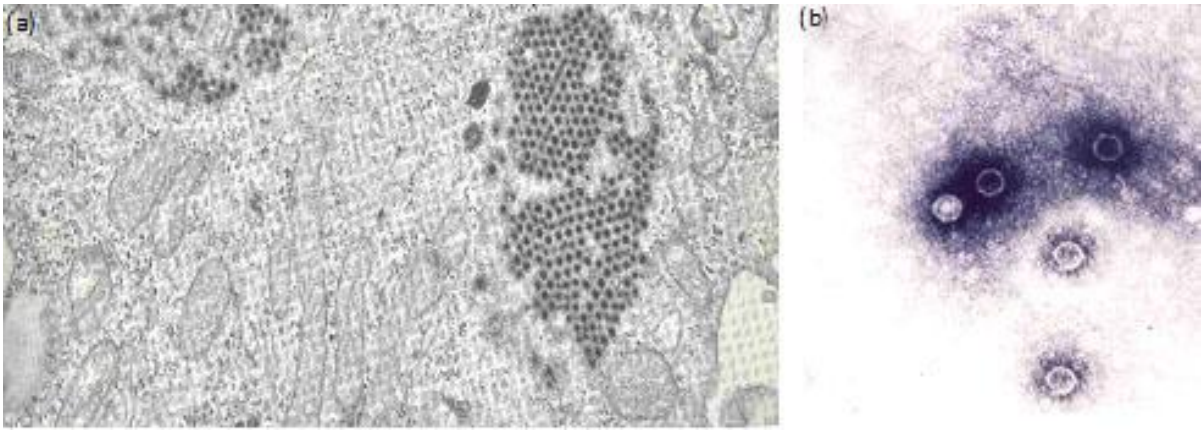

Fig. 2. (a) Electron micrograph of thin section from a reovirus infected cell superinfected with poliovirus. Many reovirus particles are present in crystal form. There is no observable poliovirus particles in the cytoplasm of infected cell (×20000). (b) Electron microscopy of the extract of poliovirus infected control cells, prepared by the negative staining technique (×120000).

used vero cells for coinfection experiments, which support growth of both viruses.

The results indicated a marked inhibition in poliovirus production in cells that were infected first with reovirus and then reinfected with poliovirus.

When the harvest of coinfected cells were used to infect another set of cell monolayer (second passage), only reovirus was produced. Electron microscopy of the infected cell lysate revealed that only reovirus particles were present. This inhibitory effect was also observed in sections of poliovirus superinfected cells where only reovirus particles were present inside the cytoplasm and no poliovirus particle was observed. In simultaneous infection of cells with reo and poliovirus it was observed that poliovirus replicated efficiently and resulted in cell lysis. The mechanism of reovirus inhibition of poliovirus growth remains to be determined.

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Table 2. Viral load of poliovirus in superinfected cells.

	Copy number of poliovirus/ml	
Experiment / **Passage Number**	**1**	**2**
Reovirus infection followed by 2 hrs superinfection with poliovirus	12	29
Control poliovirus infected cells	900,000	900,000

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