

NOTES

Failure of Rifampin to Inhibit Frog Polyhedral Cytoplasmic Deoxyribovirus Multiplication

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Received for publication 26 March 1973

Resistance of frog virus multiplication to rifampin suggests that components peculiar to cytoplasmic deoxyribonucleic acid replicating viruses (e.g., poxvirus) are not equally sensitive to rifampin.

Rifampin is an antibiotic (8) that reportedly blocks deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) polymerase activity in bacterial but not mammalian cells (6, 12, 27). The drug also interferes with multiplication of three mammalian DNA viruses, adenovirus (25), poxvirus (vaccinia) (14, 25), and African swine fever virus (5, 13), and with viability of transformed chick fibroblasts infected with Rous sarcoma virus (26). The presence of RNA polymerase activity in poxvirus cores (16) and its induction during poxvirus infection (15) have been demonstrated. It has been suggested that rifampin may inhibit poxvirus multiplication by complexing with a virus-specified polypeptide(s) (RNA polymerase?) needed for virus assembly (19, 21, 22, 24). This inhibition is rapidly reversed after removal of rifampin in the presence of inhibitors of DNA, RNA, and protein synthesis (21, 23).

Frog virus 3, a polyhedral cytoplasmic deoxyribovirus (PCDV), resembles poxvirus with respect to its intracellular site of replication and ability to be nongenetically reactivated (11). Because nongenetic reactivation is apparently confined to cytoplasmic DNA viruses (7), this group of viruses may have a common factor(s) (virion-associated RNA polymerase?) required for replication. This factor(s) may be responsible for the sensitivity of animal DNA viruses to rifampin. For these reasons, the effect of rifampin on PCDV multiplication was investigated. The results showed that PCDV multiplication in baby hamster kidney cells in suspension culture (BHK-S) was not significantly inhibited at concentrations of rifampin (100 $\mu\text{g}/\text{ml}$) which

blocked multiplication of poxvirus (rabbitpox) from 93 to 99%.

The first experiment was designed to determine the effect of different concentrations of rifampin on PCDV multiplication in BHK-S cells at 28 C, a temperature permissive for infectious virus production (9). The results (Fig. 1) indicated a slight inhibition of virus growth in the presence of 200 μg of rifampin per ml of culture medium. In cultures treated with 400 μg of rifampin per ml, the 24-h yields of virus were reduced about 90%. There was no effect on the virus latent period (Fig. 1). Similar results were obtained with monolayer cultures of BHK-S cells infected with PCDV (data not shown).

Since rifampin may interfere with transcription mediated by the transcriptase induced by poxvirus infection (19, 22), its effects on cellular and PCDV-induced RNA synthesis were determined. By 8 h after infection in the presence of 400 μg of rifampin per ml, PCDV-induced RNA synthesis (20) was suppressed by about 25% (Fig. 2A), and cellular RNA synthesis (Fig. 2B) was suppressed by about 60%. These data indicate that concentrations of rifampin which inhibit PCDV multiplication also inhibit both virus-induced and cellular RNA synthesis.

Infection with PCDV inhibits cellular DNA synthesis between 0 and 4 h; after 4 h only viral DNA synthesis is detectable (9, 18). Viral DNA, made exclusively in the cell cytoplasm (9), can be separated by cell fractionation (17) into two classes: replicating DNA (R-DNA) and encapsidated DNA (E-DNA). The next experiment was done to determine the comparative effects of rifampin on these classes of virus-induced

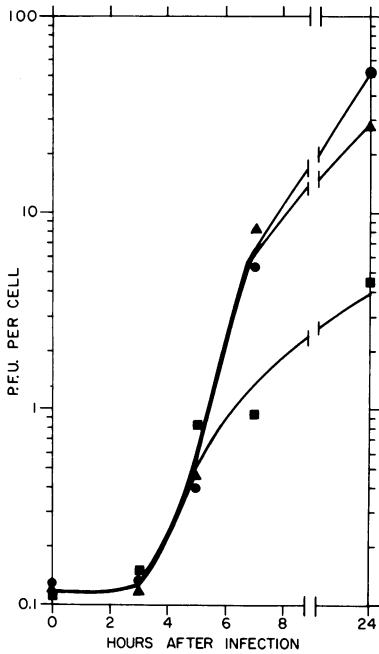


FIG. 1. Effect of different concentrations of rifampin on PCDV multiplication. BHK-S cells were infected with PCDV at a multiplicity of 10 PFU/cell (18). Cells infected at 28 C were either untreated or continuously treated with rifampin starting at 0 h (30 min after the virus attachment period). At various times after infection, duplicate cell samples (5×10^6 cells) were removed and the total virus yield (cell associated plus released virus) was measured by plaque assay (9). PFU per cell without added rifampin (●) or with 200 (▲) or 400 μg (■) of rifampin per ml was measured.

DNA and on cellular DNA synthesis. With or without rifampin treatment, viral R-DNA (Fig. 3A) and E-DNA (Fig. 3B) formation in PCDV-infected cultures started about 3 h after infection. By 8.5 h after treatment with 400 μg of rifampin per ml, however, R-DNA and E-DNA formation were inhibited by 50 and 65%, respectively (Fig. 3A and B). At 200 μg of rifampin per ml, R-DNA synthesis (Fig. 3A) was inhibited 31% and encapsidation of viral DNA (Fig. 3B) was reduced 42%. In uninfected cells, DNA synthesis (Fig. 3C) was reduced 29% with 200 μg of rifampin per ml and 59% with 400 $\mu\text{g}/\text{ml}$. Although the number of infected cells per sample and the scale for ^3H -TdR incorporation differed for virus-induced (Fig. 3A) and cellular (Fig. 3C) R-DNA, the data were interpreted in the same way. In other experiments pretreatment of BHK-S cells with rifampin (400 $\mu\text{g}/\text{ml}$) 1 h before virus infection did not quantitatively alter the observed effect of the drug on PCDV-

specific R-DNA or E-DNA formation. In addition, uninfected BHK-S cells in monolayer culture began to show degenerative changes (cell rounding and cell detachment) after incubation for 5 to 8 h at 28 C with 400 but not 200 μg of rifampin per ml. In conclusion, the toxicity of rifampin for the host cell is most likely responsible for the observed reduction in PCDV replication (Fig. 1) at the 400 $\mu\text{g}/\text{ml}$ concentration.

Because PCDV multiplication in BHK-S cells was not significantly inhibited by concentrations of rifampin (100 $\mu\text{g}/\text{ml}$) that markedly inhibited poxvirus (vaccinia) multiplication at 37 C (14, 25), an experiment was designed to determine (i) whether the resistance of PCDV replication in BHK-S cells is due to lowered permeability to rifampin and/or (ii) whether there is a temperature requirement above 28 C for demonstrating in vitro antiviral activity of the drug. BHK-S cells in monolayer cultures were infected with rabbitpox virus (5 plaque-forming units [PFU]/cell), overlaid with Eagle minimal essential medium with or without added rifampin (100–200 $\mu\text{g}/\text{ml}$), and incubated at either 28 or 37 C. At 1 or 24 h after infection, total infectious virus yield was measured by

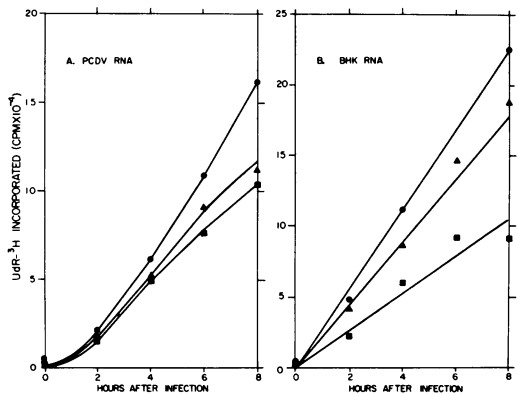


FIG. 2. Effect of different concentrations of rifampin on cellular and PCDV-induced RNA synthesis. BHK-S cells were infected with PCDV at a multiplicity of 10 PFU/cell (18) or mock infected. All cultures were incubated at 28 C and continuously labeled with ^3H -Urd (2.1 $\mu\text{Ci}/\text{ml}$; sp act 20 Ci/mmol) in the absence or presence of rifampin starting at 0 h (30 min after the virus attachment period). At various times, cell samples (5×10^6 cells) were removed to measure incorporation of ^3H -Urd into RNA (20). A, Incorporation into PCDV-induced RNA in the absence (●) or presence of 200 (▲) or 400 μg (■) rifampin per ml. B, Incorporation into cellular RNA in the absence (●) or presence of 200 (▲) or 400 μg (■) of rifampin per ml.

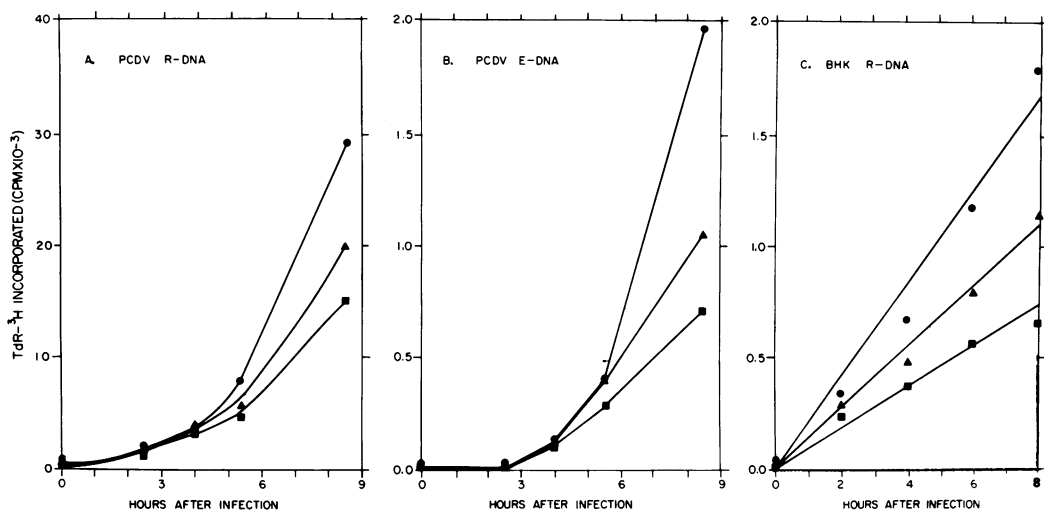


FIG. 3. Effect of rifampin on replication and encapsidation of PCDV-DNA and on replication of cellular DNA. BHK-S cells were infected with PCDV at a multiplicity of 10 PFU/cell or mock infected. All cultures were incubated at 28 C and continuously labeled with ^3H -TdR (0.26 μCi ; 1 μg of TdR/ml) in the absence or presence of rifampin starting at 0 h (30 min after the virus attachment period). At various times 10^7 infected cell samples or 5×10^6 uninfected cell samples were fractionated to measure incorporation of ^3H -TdR into replicating DNA (R-DNA) and encapsidated DNA (E-DNA). A, Incorporation into PCDV-induced R-DNA in the absence (●) or presence of 200 (▲) or 400 (\blacksquare) μg of rifampin per ml. B, Incorporation into PCDV-induced E-DNA in the absence (●) or presence of 200 (▲) or 400 (\blacksquare) μg of rifampin per ml. C, Incorporation into cellular R-DNA in the absence (●) or presence of 200 (▲) or 400 (\blacksquare) μg of rifampin per ml.

plaque assay in BHK-S cell monolayers at 37 C. Residual rifampin in the virus assay was not more than 0.005 $\mu\text{g}/\text{ml}$. The 24-h yield of rabbitpox virus at 28 C (1.9×10^5 PFU/ml) or 37 C (4.4×10^6 PFU/ml) was inhibited 93% with 100 μg of rifampin per ml and 99% with 200 $\mu\text{g}/\text{ml}$. Thus, BHK-S cells are permeable to antiviral concentrations of rifampin, and the antiviral activity is not dependent on conversion of the drug to an active form at 37 C.

In conclusion, rifampin did not selectively affect PCDV replication in BHK-S cells, because concentrations of the drug (200–400 $\mu\text{g}/\text{ml}$) that modified PCDV-induced R-DNA (Fig. 3A) and E-DNA (Fig. 3B) formation, RNA synthesis (Fig. 2A), or PCDV multiplication (Fig. 1) also caused significant modification of cellular R-DNA (Fig. 3C) and RNA (Fig. 2B) synthesis.

In contrast to my data, Heller (13) reported that rifampin (200 $\mu\text{g}/\text{ml}$) inhibits the total yield of PCDV about 90% in BHK-S cells at 26 C. It is possible that rifampin inhibition of PCDV replication reported by Heller is due to the toxic effects of the drug on BHK-S cells. In agreement with my results, Bingen and Kirn (3) reported that rifampin does not inhibit morphogenesis of PCDV in BHK cells.

Infection of host cells with PCDV markedly inhibits host cell DNA, RNA, and protein synthesis (17, 20). It is interesting to compare effects of PCDV and rifampin on cellular macromolecular synthesis. During infection with PCDV at temperatures permissive for infectious virus production (28 C), the virus selectively inhibits cellular DNA, RNA, and protein synthesis while allowing continued synthesis of virus-specified macromolecules (17, 18, 20). At temperatures nonpermissive for virus production (33–37 C) (9, 18), host-cell synthetic functions are still inhibited. PCDV has also been reported to inhibit vaccinia DNA synthesis in KB cells infected with vaccinia virus (1). A factor (capsid protein?) closely associated with PCDV may be responsible for this inhibition of viral DNA and host-cell macromolecular synthesis (1, M. Gravell, *Bacteriol. Proc.*, p. 182, 1969).

Preliminary data (Kucera, unpublished data) show that PCDV, like poxvirus, contains an RNA polymerase as an integral component of the virion. There is other evidence supporting these data (10). However, in contrast to poxvirus cores (19), *in vitro* polymerase activity in PCDV cores is inhibited by rifampin (Kucera, unpublished data). Since rifampin did not se-

lectively inhibit PCDV replication (Fig. 1) or PCDV-induced transcription in infected BHK-S cells (Fig. 2A), the inhibition of transcriptase in PCDV cores by rifampin may be due to increased accessibility of the enzyme to the drug *in vitro* as compared with infected cells.

Although PCDV may have an RNA polymerase or other component(s) peculiar to DNA viruses which replicate in the cell cytoplasm (e.g., poxvirus, 15; insect iridescent viruses, 2; African swine fever virus, 4; and lymphocystis disease virus, 28), this component(s) is not equally sensitive to rifampin. The lack of specific inhibition of PCDV multiplication by rifampin indicates that inhibition of poxvirus (25) or African swine fever virus (5) multiplication by rifampin is not a general reaction shared by all cytoplasmic DNA viruses. Further biochemical studies with PCDV should provide a useful model for comparing the basis of rifampin action against sensitive and resistant viruses.

I thank Ramona Tirey and Margie McKenzie for excellent technical assistance.

This research was supported by U.S. Public Health Service research grant CA 12382 from the National Cancer Institute. A portion of this work was completed in the Laboratories of Virology and Immunology, St. Jude Children's Research Hospital, Memphis, Tennessee and supported by Public Health Service grant CA 07055 from the National Cancer Institute and by ALSAC.

I am grateful to A. Granoff and D. Kingsbury for helpful suggestions.

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