Inhibition by Rifampin of African Swine Fever Virus Replication in Tissue Culture

A. H. DARDIRI, H. L. BACHRACH, AND E. HELLER

*Plum Island Animal Disease Laboratory, Agricultural Research Service, Greenport, New York 11944,
and Department of Virology, Hebrew University, Hadassah Medical School, Jerusalem, Israel*

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Vaccinia virus and African swine fever virus are deoxyribonucleic acid viruses of cytoplasmic origin. The fact that rifampin inhibits the replication of the former virus led to an investigation of its effect on African swine fever virus. The virus used was cytopathogenic to a PK-15 cell line, hemadsorbing in pig leukocyte cultures and lethal to pigs. Rifampin clearly inhibited the multiplication and cytopathogenicity of the virus in PK-15 cells. There was a 1- to 5-log reduction in virus titer depending upon the rifampin concentration, the multiplicity of infection, and the time after infection. Inhibition was greatest at a concentration of 200 μg of rifampin/ml. The drug was not viricidal per se, and the inhibition of virus replication was not due to the cell-granulating effect of rifampin since cultures which were transiently pre-treated for long as 90 hr with 200 μg of drug/ml supported viral replication to the same degree as untreated cultures.

The antibiotic rifampin (RMP) inhibits the growth of bacteria (3, 9), trachoma agent (1, 2, 4), bacteriophage (7), and vaccinia virus (4, 8, 12). Its inhibiting action on bacteria appears to be on a deoxyribonucleic acid (DNA)-dependent ribonucleic acid (RNA) polymerase. The first evidence of its activity on vaccinia virus in tissue culture indicated an interference in the synthesis of viral protein late in the growth cycle (4). Since then, it has been shown that RMP blocks the induction of four virus-associated enzymes (nucleotide phosphohydrolase, RNA polymerase, and two deoxyribonucleases) in L cells infected with wild-type vaccinia virus (10).

African swine fever virus (ASFV), like vaccinia virus, is a DNA virus of cytoplasmic origin (5, 6). This suggested that ASFV might display a sensitivity to RMP similar to that of vaccinia virus. The purpose of this investigation was to determine the sensitivity of ASFV to RMP, thereby gaining additional insight on the selective inhibition by RMP for mammalian viruses and on the potential of the compound as an antiviral agent for African swine fever.

MATERIALS AND METHODS

Virus. ASFV, Tengani strain, had been adapted to cell culture by 45 passages in pig leukocyte cultures followed by 13 passages in a pig kidney cell line, PK-15. It produced a cytopathogenic effect (CPE) in PK-15 cells and specific hemadsorption in leukocyte cultures and was fatal to pigs. Crude virus harvests

contained 7.8 $\log_{10}$ 50% tissue culture infectivity dose₅₀ (TCID₅₀)/0.5 ml for PK-15 cells.

Drugs. RMP was obtained from Lepetit Laboratories, Milan, Italy, and Dow Chemical Co., Zionsville, Ind. A stock solution of 200 μg /ml was prepared by solution of the drug in 0.1 N HCl and subsequent dilution with maintenance medium.

Cell culture. Monolayer cultures of the PK-15 cell line were prepared in 2 oz (ca. 60 ml) Falcon plastic flasks with Eagle's minimum essential medium (MEM) and 5% fetal calf serum.

Determination of inhibitory drug concentrations. The effect of RMP on ASFV replication was determined by the addition of graded doses of the drug to PK-15 cultures which had received doses of virus varying by 10-fold. Portions of virus (0.5 ml), decimally diluted 10^0 to 10^{-9} in MEM containing 2% calf serum (maintenance medium), were added in duplicate to a series of cultures. After 45 min at 37 C, each culture was washed thrice with maintenance medium. Six milliliters of maintenance medium without RMP and with RMP at 10, 50, 100, 150, and 200 μg /ml was then added, and the incubation was continued. Each culture was observed daily for a CPE, and TCID₅₀ were computed at appropriate times by the method of Reed and Muench (11).

Determination of viricidal effect of RMP. To determine the viricidal effect, if any, of RMP on ASFV, the virus was decimally diluted in maintenance medium with and without 200 μg of the compound per ml. After incubation for 5, 30, and 60 min at 28 C, 0.5 ml of the treated virus was pipetted into sets of triply washed cultures. Control cultures were treated similarly with maintenance medium containing 200 μg of the compound per ml. After 60 min incubation at

37 C, maintenance medium was added, and incubation was continued for 6 days at which time all cultures were examined for CPE.

Toxicity control. As a control on drug toxicity, sets of noninfected cultures with and without graded doses of RMP were observed for cytopathic changes over a 6-day period. A more quantitative control consisted of measuring the degree to which cultures recovered their sensitivity to virus after treatment for 24 to 90 hr with 200 μ g of RMP per ml (Table 2). In such tests, the cultures were usually inoculated with virus immediately after removal of the RMP by three washes with maintenance medium; in two tests in which drug was present for 84 or 90 hr, fresh RMP-free medium was also added to the washed cultures 6 hr prior to inoculation with the decimally diluted virus doses. Titrations of virus in cultures not previously exposed to the drug, with and without 200 μ g of RMP per ml added 45 min after infection, were included as well as uninfected cultures with and without the compound.

RESULTS

The effect of RMP on ASFV multiplication during a 6-day observation period is presented in Table 1. Virus titers were reduced as the concen-

TABLE 1. *Mean cytopathogenic titers of African swine fever virus (ASFV) in PK-15 cells in the presence of rifampin*

Time post-infection (day)	Titer of ASFV ^a in presence of rifampin (μ g/ml)					
	0	10	50	100	150 ^b	200
1	1.5	1.0	1.0	1.0	1.0	1.0
2	5.5	6.0	6.0	5.0	3.0	2.5
3	7.9	8.0	7.0	6.0	4.0	3.0
4	8.0	8.0	7.0	7.0	5.0	3.0
5	8.0	8.0	7.0	7.0	6.0	3.0
6	8.0	8.0	7.0	7.0	7.0	3.0

^a Log₁₀ 50% tissue culture infectivity dose/0.5 ml.

^b Assays in quadruplicate; other concentrations, assays in duplicate.

tration of the compound was increased above 10 μ g/ml. At 50 μ g/ml and higher concentrations, RMP clearly inhibited the multiplication and CPE of ASFV in PK-15 cells. There was a 1- to 5-log reduction in virus titer depending upon the RMP concentration, the amount of virus in the inoculum, and the interval after infection. Inhibition was greatest at 150 and 200 μ g of the compound per ml. At 150 μ g/ml, the main effect was to slow down the infectious process. The 4-log depression in titer at 3 days diminished to only a 1-log depression at 6 days. At 200 μ g/ml, a 5-log depression in titer persisted from the 3rd day on.

The test of the effect of the drug on uninfected cell cultures indicated that monolayers exposed to 200 μ g of RMP per ml remained normal up to 72 hr after addition of the medium. Discernible granulation of the cell monolayer was apparent at 96 hr and remained constant thereafter without detachment of cells until the end of the observation period. The effect on the cell monolayer was correlated with the concentration of the drug. At 100 and 150 μ g of RMP per ml, cell granulation was less evident. At 50 and 100 μ g of RMP per ml, where little or no granulation was observed, the drug still depressed the ultimate virus titer by 1 log.

Furthermore, Table 2 shows that the cell-granulating effect of 200 μ g of RMP per ml had no effect upon the sensitivity of PK-15 cells to ASFV. Cell layers that became granulated during 84 and 90 hr of transient pretreatment with 200 μ g of RMP per ml were as sensitive to virus as cells with no pretreatment with RMP or with 24 to 72 hr of transient pretreatment where no granulation was observed. Granulated cultures did not require 6 hr of treatment with RMP-free medium to recover their sensitivity to ASFV.

In the trials where graded dilutions of the virus were exposed to 200 μ g of RMP per ml for 5, 30, and 60 min before pipetting into cultures,

TABLE 2. *Mean cytopathogenic titers of African swine fever virus (ASFV) in PK-15 cells after transient exposure to rifampin (RMP)^a*

Expt	Cultures without preexposure		Titers of ASFV ^b at hr of transient preexposure of culture to 200 μ g of RMP/ml				
	0	200 ^c	24	48	72	84	90
1	8.2 ^d	3.2	7.8	8.2	8.0		
2	8.1 ^d	3.0				8.0, 8.0	8.0, 8.0
3	8.1 ^d	3.5	7.8	7.8	8.0		

^a Amount: 200 μ g/ml.

^b Log 50% tissue culture infectivity dose/0.5 ml.

^c The 200 μ g of rifampin/ml was added 45 min after infection with virus.

^d Mean of four to six titrations.

exposed and unexposed virus yielded titers of approximately $10^{7.2}$ /0.5 ml, indicating that RMP is nonviricidal.

DISCUSSION

The compound RMP clearly inhibited the CPE and multiplication of ASFV. The degree of inhibition was found to be a function of the drug concentration and the length of exposure. The level of the compound at which marked reduction occurred in the yield of ASFV was higher than that at which most other microorganisms are inhibited (2-4, 8, 9, 11, 12). Sensitive vaccinia virus strains are inhibited with 100 μ g of the compound per ml (10, 12), which is two-thirds or half the concentration with inhibited ASFV.

The marked inhibition of ASFV replication by RMP at 200 μ g/ml does not appear to be due to the cell-granulating effect of high concentrations of the compound. Inhibition was already maximal at 72 hr at which time control cells treated with 200 μ g/ml of drug alone still remained normal in microscopic appearance. Also, cell layers with 24 to 90 hr of transient pretreatment with 200 μ g of RMP per ml supported viral replication to the same extent as untreated cultures.

These results, taken together, demonstrate that RMP at 200 μ g/ml inhibits specifically and markedly the replication of ASFV in PK-15 cells. Additional work will be required to determine similarities in the mechanisms of RMP inhibition of vaccinia virus (4, 10) and ASFV replication. However, the common sensitivity of both viruses to this compound, their DNA content and cytoplasmic replication, as well as the ap-

parent association of ribosomes at the periphery of developing ASF virions (5) indicates that a specific DNA-dependent RNA polymerase is associated with ASFV.

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