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# Inhibition of replication of rinderpest virus by 5-fluorouracil

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**Abstract**

5-Fluorouracil (5FU), an analogue of uracil, was found to inhibit the production of infectious particles of rinderpest virus (RPV) in Vero cells (African green monkey kidney cells) by 99%, at a concentration of 1 $\mu$g/ml. The levels of individual mRNA specific for five of the virus genes were also reduced drastically, while the level of mRNA for a cellular housekeeping gene—glyceraldehyde-3-phosphate dehydrogenase (GAPDH)—was unaltered by fluorouracil treatment of infected cells. Both virus RNA and protein synthesis showed inhibition in a dose-dependent manner. The virions which budded out of 5-fluorouracil-treated cells also contained reduced amounts of virus proteins compared with virus particles from untreated cells.

*Keywords:* Rinderpest virus; 5-Fluorouracil; Virus transcription; Antiviral drug

## 1. Introduction

The anti-metabolite 5-fluorouracil (5FU) has been used to study its effect on biosynthetic events in virus-infected cells. 5FU has at least two important modes of action: (i) it is converted in cells to fluorodeoxyuridine monophosphate (FdUMP), which inhibits the enzyme thymidylate synthetase (Danenberg, 1977; Danenberg and Danenberg, 1978; Danenberg and Lockshin, 1982), leading to inhibition of DNA synthesis; (ii) fluorouracil triphosphate (FUTP) can incorporate into RNA, leading to alteration of RNA function and metabolism (Spiegelman et al., 1980). 5FU replaces uracil in all types of RNA, and alteration in structure and function of tRNA (Madan Kumar and Nayak, 1990; Vulimiri and Nayak, 1993, 1994), rRNA (Herrick and Kufe, 1984) and mRNA (Will and Dolnick, 1989; Wu and Dolnick, 1993) caused by 5FU can affect the translational machinery, leading ultimately to cytotoxicity.

The effect of 5FU on DNA viruses has been examined in detail and in most cases, the synthesis of virus-specific proteins in the absence of mature virus formation has been shown to occur in the presence of 5FU. In the case of pseudorabies virus (Ben-Porat et al., 1967) and SV40 virus (Melnick and Rapp, 1965), empty capsids were seen, but even so, abundant virus-specific proteins were detected in treated infected cells. In the case of infectious canine hepatitis virus grown in vitro (Altera and Moulton, 1966), 5FU abolished the

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production of infectious virus particles, whereas infected cells showed reduced hemagglutination and virus-specific antigen production. 5FU inhibited the multiplication of the papilloma virus (Brillhart, 1990), herpes virus and vaccinia virus (Dragun et al., 1990).

In the case of RNA phage MS2, substitution of uracil by 5FU produced inactive particles (Shimura and Nathans, 1964) and such substituted defective viruses were shown to carry molecules of stable 6S RNA, tRNA of *E. coli*, together with a broken viral RNA molecule in its capsid (Shiba and Saigo, 1982). However, in the case of poliovirus (Munyon and Salzman, 1962) and Tobacco Mosaic virus (Gordon and Staehelin, 1959) 5FU incorporation does not always alter the function of RNA, whereas there are reports demonstrating an increased mutation rate of these viruses in the presence of 5FU (Cooper, 1964; Davern and Bonner, 1958). 5FU has been used as a mutagen for a large number of animal RNA viruses (Bussereau et al., 1982; Cosby et al., 1985; Caplan et al., 1985; Chui et al., 1986; Agut et al., 1989; Takehara et al., 1989) and attenuated mutant viruses have been checked for their immunogenicity and pathogenicity for use as candidate vaccines (Tolson et al., 1990; Hubbard et al., 1991). We report here the antiviral action of this drug using rinderpest virus as a model system.

Rinderpest virus (RPV) is the causative agent of an acute, febrile disease of cattle and wild bovids, and causes high mortality. RPV belongs to the genus Morbillivirus in the family Paramyxoviridae (Barrett et al., 1991). It is a negative stranded, enveloped RNA virus. From the 3′- to 5′-end, the genomic RNA encodes virus proteins in the following order: nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin protein (H) and polymerase protein (L). The genomic RNA is encapsidated with N protein to form a helical structure called nucleocapsid. P and L proteins are associated with nucleocapsid. The envelope of the virus contains H and F proteins which are responsible for cell attachment and cell fusion functions. In between two gene sequences, there is a stop signal, an intergenic sequence and a start signal for the next gene. The polymerase falls off from the template at the end of each gene, thus proximal N gene transcripts are synthesized more than distal L gene transcripts. After sufficient protein synthesis has taken place, replication starts through a full-length positive sense genomic RNA intermediate that serves as a template to produce negative sense genomic RNA. The genome of these groups of viruses contain large stretches of uracil residues at the 3′-, 5′-ends and intergenic regions (Galinski and Wechesler, 1991) and 5FU could effectively replace these uracil residues and thereby bring about alterations in the synthesis of mRNA transcripts, antigenomic and genomic RNA.

Previously we have demonstrated that 5FU causes marked alterations in the structure of the nucleocapsid complex of RPV (Ghosh et al., 1994). This communication presents data on the antiviral activity of 5FU against RPV. Here, we provide evidence to show that 5FU significantly inhibits the multiplication of RPV in Vero cells (African green monkey kidney cells). The effect of this drug on the transcription, translation and virus multiplication is described.

## 2. Materials and methods

### 2.1. Cell cultures and virus

African green monkey kidney cells (Vero cells) were obtained from the National Facility for Animal Tissue and Cell Culture, Pune, India and were grown in Eagles minimum essential medium (E-Mem) containing 4% fetal bovine serum and antibiotics, streptomycin (50 $\mu$g/ml) and benzylpenicillin (100 U/ml). The virus strain used was RBOK RPV Kabete 'O' vaccine strain (tissue culture adapted), and was obtained from the Institute of Animal Health and Veterinary Biologicals, Bangalore.

### 2.2. Reagents and radioisotopes

5-Fluorouracil (5FU) was obtained from Sigma Chemical Co., St. Louis, MO, USA.

Radiochemicals: $\alpha$-$^{32}$P dATP (sp. act. 3000 Ci/mmol) and $^{35}$S- methionine (sp. act. 1000 Ci/mmol) were obtained from the Bhabha Atomic Research Centre, Bombay.

Antisera: RPV hyperimmune sera (polyclonal, rabbit raised) was obtained from the Institute for Animal Health, Pirbright Laboratory, UK.

### 2.3. Cell viability test

Viability of cells was checked according to Yang et al. (1988) to determine the concentration of 5FU which is not toxic to host cells, but has antiviral effects. A range of concentrations of 5FU was added to confluent monolayers of Vero cells in 24-well plates and the cells were maintained in the presence of 5FU for 3 days. Viable cell counts of each culture were determined by the trypan blue dye exclusion method.

### 2.4. Cell growth rate

The assay of growth rate of Vero cells in the presence of 5FU was carried out according to the method of Hu and Hsiung (1989). Concentrations of drug similar to that used for the cell viability assay were used to monitor the growth rate. 5FU was added to Vero cells in 24-well plates 24 h after seeding. The treatment was continued for 3 days and each day the cell viability was monitored. The 5FU concentration required to inhibit growth rate by 50% was calculated using 5FU-free control cultures as reference.

### 2.5. Viability and infectious particle assay

Inhibition of virus-induced cytopathic effect (CPE) was determined by the method of De Clercq et al. (1980) and Field et al. (1986). Vero cells were seeded in 96-well cell culture dishes. When a monolayer was formed in each well, cells were infected with a constant dose of 50 $TCID_{50}$ (tissue culture infective dose 50) units of virus. After 1–2 h of virus adsorption at 37°C, virus was removed and E-MEM containing various concentrations of 5FU was added to the cultures. For each drug concentration, there were quadruplicate cultures. RPV-induced CPE was recorded each day until all the quadruplicate cultures in the virus control (i.e. cultures without 5FU) showed CPE. The 50% effective concentration ($EC_{50}$) for 5FU was determined by the concentration that inhibited CPE in half of the quadruplicate cultures.

To determine the effect of 5FU on the release of infectious RPV particles, the methodology described by Hu and Hsiung (1989) was followed. Vero cells were grown in 24-well dishes and infected with RPV (at 100 $TCID_{50}$ units/well). After 1 h of adsorption, the cells were washed and treated with a range of 5FU concentrations at 0 h post infection. One set of wells served as untreated control. At 48 h post infection, when CPE had progressed well in cultures with virus control, supernatant fluids were collected from treated and untreated wells and checked for released virus particles by the $TCID_{50}$ method (Reed and Muench, 1938).

### 2.6. Cell extract preparation

Confluent monolayers of Vero cells grown in 35-mm dishes were infected with RPV and different concentrations of drug were added at 0 h post infection. At 48 h post infection, the monolayers were washed twice with chilled phosphate buffered saline (PBS). The cells were scraped into 200 $\mu$l of PBS and sonicated three times with a pulse of 30 s at maximum amplitude. The nuclei were removed by low speed centrifugation at 4°C. The supernatant which constituted the soluble proteins was aliquoted and stored at $-70$°C.

The total protein content of each sample was determined according to Lowry et al. (1951) and total virus protein present in each was measured by indirect ELISA using RPV hyperimmune sera (Barrett et al., 1989).

### 2.7. Isolation of RNA and dot blot hybridization

Confluent monolayers of Vero cells, grown in 25-$cm^2$ tissue culture flasks, were infected with RPV (at 0.01 $TCID_{50}$ units/cell) and different concentrations of drug were added at 0 h post infection. For each drug concentration, cells equivalent to two 25-$cm^2$ flasks were taken. At 48 h post infection, when CPE was pronounced, the monolayers were washed twice with chilled PBS. The RNA was extracted by acid guanidine isothiocyanate single step isolation method (Chomzyn-

sky and Sacchi, 1987). Total RNA (25 μg) was blotted onto Hybond N membrane, and fixed by UV crosslinking. The membrane was prehybridized for 4 h in 50% formamide, 5 × SSC, 5 × Denhardts, 50 mM sodium phosphate and 100 μg/ml calf thymus DNA, and hybridized for 16 h at 42°C after addition of $^{32}$P-labelled probes specific for each gene. Washing was done with 2 × SSC and 0.1% sodium dodecyl sulphate (SDS) for 15 min three times, with 0.5 × SSC and 0.1% SDS at 60°C for 10 min twice and a final wash was done in 0.2 × SSC. The blots were exposed to autoradiographic films for 2 days.

### 2.8. Preparation of cDNA probes

RPV gene-specific cDNA cloned into plasmid pUC13, pGem or bluescript KS$^+$ contained the following RPV (RBOK vaccine strain) genes: N (D-74), M (C-6), F (pF23), H (RBH 3.4) (generously provided by Dr. T. Barrett, Institute of Animal Health, Pirbright, UK). A full-length P gene (p3-35) was isolated beforehand in the laboratory from a cDNA library of RPV RBOK vaccine strain. A clone carrying the full-length glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene from rat muscle (Fort et al., 1985) was a kind gift of Dr. Kondaiah, Centre for Reproductive Biology and Molecular Endocrinology, Indian Institute of Science, Bangalore. The inserts were released from the clones by using appropriate restriction enzymes, and then purified. They were used to make radiolabelled probes, using $\alpha$-$^{32}$P dATP by the method of Feinberg and Vogelstein (1983), and purified by Sephadex G50 spun column chromatography.

### 2.9. Northern blot analysis of RNA

For northern blots, equal amounts of total RNA (25 μg) from normal and treated infected cells were run on a formaldehyde agarose gel in MOPS buffer system according to the method described in Sambrook et al. (1989), blotted on to Hybond N membrane and hybridized to different gene-specific probes, as described previously. After exposing the blots to the X-ray films, the signals were scanned in a LKB laser densitometer and quantified. Since a 5FU concentration of 1 μg/ml reduced production of infectious virus particles by 99%, this drug concentration was used for this experiment and all subsequent experiments.

### 2.10. Preparation of $^{35}$S-methionine labelled cell extract

Vero cell monolayers grown on 35-mm dishes were infected with RPV, or RPV with 1 μg/ml 5FU added at 0 h post infection and FU-substituted RPV (RPV grown in 1 μg/ml drug concentration). 48 h post infection, when visible CPE was seen, the monolayers were washed with PBS and incubated with methionine-free medium (Sigma) for 1 h. At the end of this period, the culture medium was removed and fresh methionine-free medium was added with $^{35}$S-methionine (500 μCi/ml). Cells were further incubated for 2 h and after the incubation period, washed with cold PBS twice and lysed by the addition of RIPA buffer (2% Triton × 100, 1% deoxycholate, 0.1% SDS, 1 mM Tris-HCl (pH 7.8), 0.15 M NaCl, 1 mM PMSF) (Bhavani et al., 1989). The lysed cell extract was incubated on ice for 20 min and then pelleted first at 1000 g for 5 min followed by centrifugation at 60 000 g for 45 min. The supernatant was immunoprecipitated with RPV hyperimmune sera for the analysis of whole virus proteins. The precipitated immune complexes were separated by SDS-PAGE (polyacrylamide gel electrophoresis) and the gel was fluorographed.

### 2.11. Purification of virus

Vero cells were infected with 0.01 $TCID_{50}$ units/cell in the absence or presence of 1 μg/ml of 5FU, added at the time of infection. After 72 h, the virus released into the supernatant was collected and pelleted at 100 000 g in a Beckman ultracentrifuge using SW28 rotor for 1 h. The pellet was resuspended in PBS and layered over 15% sucrose (w/w), pelleted onto a 60% sucrose cushion in TE (pH 7.5) by centrifugation at 110 000 g in a Beckman SW41 rotor for 1 h. The opalescent virus band at the 15%–60% interphase was collected,

diluted with PBS and pelleted again at 100 000 g. The virus pellet was resuspended in a small volume of PBS, the protein concentration was determined by Lowry's method, (Lowry et al., 1951) and stored in aliquots at −70°C.

## 3. Results

### 3.1. Effect of 5FU on the multiplication of RPV in Vero cells

A range of concentrations of 5FU starting from 0.1 μg/ml to 10 μg/ml was used to study the inhibition of virus multiplication. The $EC_{50}$ value (concentration of 5FU required for inhibition of CPE in 50% of cultures) was 0.5 μg/ml. The concentration of the drug required for 50% inhibition of virus-specific RNA synthesis was 1.2 μg/ml. This concentration was determined by probing total RNA from infected and infected treated (1–5 μg/ml) cells with $^{32}P$-labelled cDNA probe specific for the N gene. Similarly, the value for 50% inhibition of virus-specific protein synthesis was 1.5 μg/ml. The dose of drug needed to produce toxicity in 50% cells was found to be 16 μg/ml. The concentration of the drug required for 50% inhibition of growth of Vero cells ($IC_{50}$) was 17 μg/ml.

These results reveal that the dose of 5FU required for 50% inhibition of CPE is 2–3-fold lower than that required for 50% inhibition of virus-specific RNA and protein synthesis. It is also clear that 5FU shows selective antiviral activity and the calculated selectivity index ($IC_{50}/EC_{50}$) is 34, using an $EC_{50}$ value of 0.5 μg/ml for the inhibition of CPE of the virus. Furthermore, 1 μg/ml of 5FU inhibits the production of infectious virions by greater than 99%.

### 3.2. Effect of 5FU on RPV gene-specific mRNAs

The amounts of individual gene-specific mRNAs, i.e. N, P, M, F, H, present in cells treated with 5FU were then measured. 5FU treatment reduced the level of N and P mRNA by 19% and 18.5%, respectively, whereas the levels of downstream gene-specific transcripts (M, F and H) were greatly reduced (67%, 86% and 90%, respectively). Fig. 1a, shows the Northern blot analysis of all mRNAs from infected and infected treated cells. However, the level of mRNA for a cellular housekeeping gene, GAPDH, showed no reduction in 5FU-treated cells (Fig. 1b).

### 3.3. Effect of 5FU on the synthesis of virus proteins in infected cells

The effect of 5FU treatment on the synthesis of virus-specific proteins in infected cells was studied by pulse-labelling infected cells which were treated with 5FU at the time of infection, followed by immunoprecipitation with hyperimmune serum against purified virus and SDS-PAGE analysis followed by fluorography. Virus proteins derived from infected 5FU-treated cells (Fig. 2, lane 2) showed severe inhibition of synthesis of all the virus proteins compared to untreated, infected cells (lane 1). In order to see whether such virus (grown in the presence of 5FU) could produce virus-specific proteins upon infection of Vero cells in the absence of 5FU, the same experiment was repeated except that 5FU-substituted RPV was used and no additional 5FU was present during infection and labelling. The result showed more pronounced inhibition of synthesis of all the virus-specific proteins (lane 4), such that they could not be picked up by hyperimmune serum, as compared with the control (lane 3). The actin protein, which usually coprecipitates with virus proteins in the immunoprecipitation of infected cell extracts with polyclonal antisera, served as an internal marker and showed no change at all in either control or 5FU-treated cells.

### 3.4. Effect of 5FU on virion proteins from purified virus

Normal and treated (grown in the presence of 1 μg/ml 5FU) virus (50 μg) was disrupted and the proteins were electrophoresed on a SDS-polyacrylamide gel. Silver staining of the gel showed reduction in the amounts of all virus-specific proteins with an apparent loss of P protein in treated virions (Fig. 3). The lower molecular weight of the nucleocapsid protein, as seen in

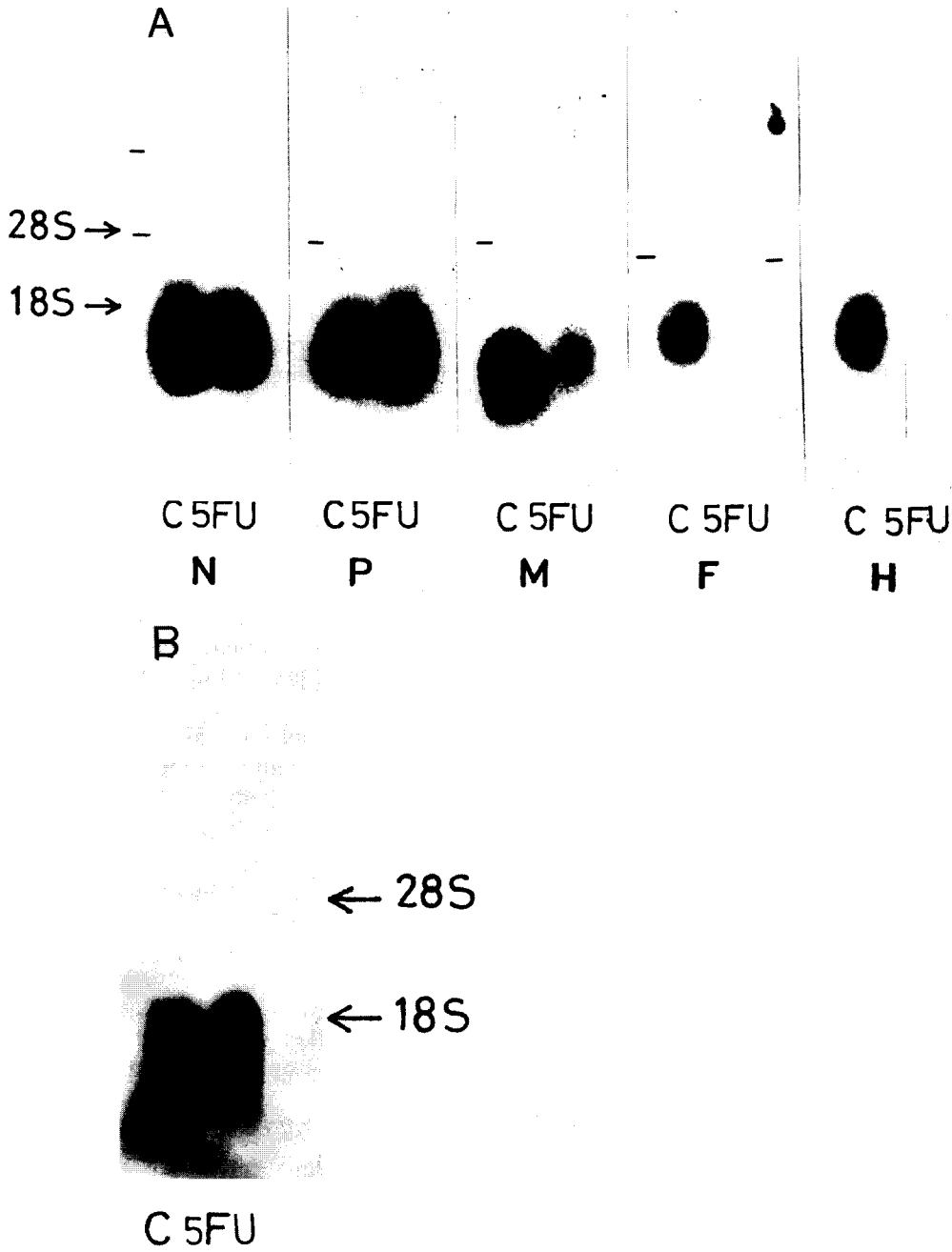


Fig. 1. Effect of 5FU on RPV gene-specific mRNA synthesis. Total RNA from infected cells (C), and from infected cells treated with 5FU at a concentration of 1 $\mu$g/ml (5FU), were run on a formaldehyde agarose gel, transferred onto an N membrane and probed with (a) different gene-specific cDNA probes, N, P, M, F, H or with (b) a cellular housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

both the normal and the 5FU virion lane, is a proteolytic degradation product of the N protein of 48 kd, which is frequently seen in all Paramyxoviruses.

## 4. Discussion

We have earlier reported (Ghosh et al., 1994) that 5FU alters the structures of rinderpest virus and viral nucleocapsids. The striking morphological differences noted were the loss of glycoprotein projections on the virion envelope and condensed nucleocapsid structure, exhibiting 'beads on string' appearance in place of the normal herring-bone structure. These results suggested two lines of investigation, (a) the molecular basis of the morphological change and (b) altered biochemical consequences resulting from structural alterations. The present study was aimed at understanding the second part.

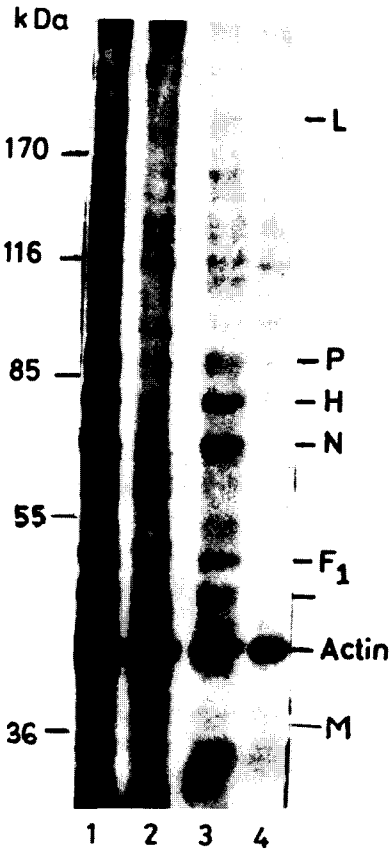


Fig. 2. Effect of 5FU on RPV protein synthesis. Infected Vero cells (lanes 1, 3), infected Vero cells incubated with 5FU at a concentration of 1 μg/ml (lane 2) and FU-RPV-infected Vero cells without any 5FU (lane 4) were labelled with $^{35}$S-methionine, cell extract was prepared, immunoprecipitated with RPV hyperimmune serum, run on SDS-PAGE, and fluorographed.

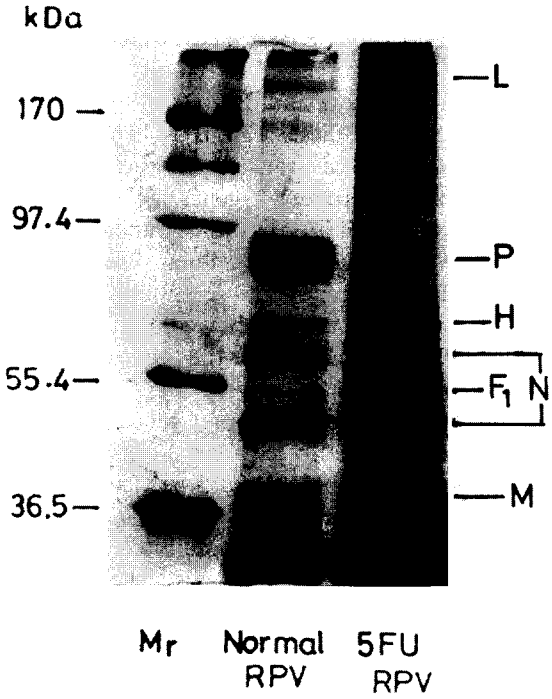


Fig. 3. Effect of 5FU on virion proteins from purified virus. Equal quantities of purified normal and FU-treated RPV were run on an SDS gel and were silver stained.

It was of importance to know that the biochemical and morphological changes produced were not due to the toxic effects of 5FU on Vero cells. The $IC_{50}$ for Vero cells was found to be 17 μg/ml whereas the $EC_{50}$ value for virus growth was 0.5 μg/ml. Therefore, a therapeutic index (selectivity index for virus) of 34 was obtained which is a reasonable window between toxic and therapeutic activity.

In the present study, all the experiments were carried out at a 5FU concentration of 1 μg/ml—1/17th of toxic concentration. The levels of a few cellular housekeeping genes like actin and GAPDH have been routinely monitored to ensure that the effect of the drug is due to the direct action on the virus and not due to indirect effects through inhibition of cellular functions.

Our results clearly demonstrate that 5FU inhibits replication of RPV and the virus stocks prepared from 5FU-treated cells are only 1% as

infectious as the virus from untreated cells. The RNA genome of RPV has stretches of U residues in the intergenic, start and end of individual genes (Baron and Barrett, 1995). Substitution of 5FU in these regions is likely to alter the function of the transcribed and translated products from structural genes. We have observed severe inhibition in the transcription of viral genes, compared to cellular housekeeping genes, after 5FU treatment.

Northern blot analysis showed that transcription of different genes in the genome of the virus is inhibited to varying degrees whereas that of cellular housekeeping genes like actin and GAPDH is unaffected. An alternative explanation for this inhibition could be that a cellular transcription factor necessary for rinderpest replication was selectively inhibited by 5FU. However, this is not likely because, to date, there is no evidence that such factors are involved in paramyxovirus replication (Moyer and Horikami, 1991). Although paramyxoviruses do require host stimulatory factors such as actin or tubulin, as has been shown by different groups of workers in the case of respiratory syncytial virus (RSV), human parainfluenza virus type 3 (HPIV3), measles virus (MV), as well as by us in the case of RPV (De et al., 1991; Moyer et al., 1990 and Ghosh et al., 1995), these proteins have been thought to act as scaffolding factors in holding virus transcription machinery together. Although theoretically it is possible that host cellular factors are responsible for reducing the levels of virus transcription as a consequence of inhibition by 5FU, one such cellular protein, actin, is not altered by 5FU treatment at the concentration used in the present work.

The same mechanism of 5FU incorporation into RNA will occur in the case of cellular transcripts, but at much higher doses. In fact, this may be responsible for the decrease in cell viability at higher doses of the drug. But at a concentration as low as 1 $\mu$g/ml, the effects are virus-specific. Moreover, with cells, the effect of this drug would be linear, i.e. the effect of substitution of 5FU into an RNA will be confined to that RNA only and perhaps will be reflected on the respective protein products whereas substitution into viral RNA will be transmitted to generations of viruses through cycles of replication. A single base substitution in genomic RNA or antigenomic RNA will be amplified many times over through cycles of replication, as a result of which we see alterations in the virus structure and its corresponding nucleocapsids.

Immunoprecipitation of newly synthesized proteins and their quantification, as well as the steady-state levels of virus proteins in the treated cells, were markedly reduced on FU treatment which may be a consequence of reduced levels of virus transcripts in the cell. Analysis of proteins in pure virus released from FU-treated cells has shown the apparent absence of the P protein. Considering that the inhibition of transcription of the P gene is only 20%, this results appears surprising. However, it is possible that all the synthesized P protein may not be incorporated into the virus nucleocapsid. Altered association of proteins with RNA has been shown in the case of ribosomes in FU-treated bacterial cells (M. Joshi and R. Nayak, unpublished).

In summary the well known anti-cancer agent 5FU exhibits antiviral properties against a RNA virus of considerable economic importance. The results of this study provide a rationale for further investigations of the possible antiviral action of 5FU against other RNA viruses. Any base and nucleoside analogues sharing properties with 5FU are also candidate antiviral agents. There are not many antiviral drugs available against RNA viruses (positive strand, negative strand and retroviruses) which are the etiological agents of many human and animal diseases. The use of established drugs of known molecular mechanism of action, with or without combination with other antiviral agents, would be useful in the care and management of many of these diseases.

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## References

Agut, H., Kean, K.M., Fichot, O., Morasco, J., Flanegan, J.B. and Girard, M. (1989) A point mutation in the poliovirus polymerase gene determines a complementable temperature-sensitive defect of RNA replication. Virology 168, 302–311.

Altera, K.P. and Moulton, J.E. (1966) Effects of 5-fluorouracil on infectious canine hepatitis virus infection of dog kidney cells. Proc. Soc. Exp. Biol. Med. 121, 157–161.

Baron, M.D. and Barrett, T. (1995) The sequence of the N and L genes of rinderpest virus, and the 5′ and 3′ extragenic sequences: the completion of the genome sequence of the virus. Vet. Microbiol. 44, 175–186.

Barrett, T., Belsham, G.J., Shaila, M.S. and Evans, S.A. (1989) Immunization with a vaccinia recombinant expressing the F protein protects rabbits from challenge with a lethal dose of rinderpest virus. Virology 170, 11–18.

Barrett, T., Subbarao, S.M., Belsham, G.J. and Mahy, B.W.J. (1991) The molecular biology of the morbilliviruses. In: D.W. Kingsbury (Ed), The Paramyxoviruses, pp. 83–100. Plenum Press, New York.

Ben-Porat, T., Kaplan, A.S. and Tennant, R.W. (1967) Effect of 5 fluorouracil on the multiplication of a virulent virus (pseudorabies) and oncogenic virus (polyoma). Virology 32, 445–456.

Bhavani, K., Karande, A.A. and Shaila, M.S. (1989) Preparation and characterization of monoclonal antibodies to nucleocapsid protein N and H glycoprotein of rinderpest virus. Virus Res. 12, 331–348.

Brillhart, J.R. (1990) The diagnosis and treatment of genital human papillomavirus lesions. Indiana Med. 83, 652–656.

Bussereau, F., Benejean, J. and Saghi, N. (1982) Isolation and study of temperature-sensitive mutants of rabies virus. J. Gen. Virol. 60, 153–158.

Caplan, H., Peters, C.J. and Bishop, D.H. (1985) Mutagen-directed attenuation of Rift valley fever virus as a method for vaccine development. J. Gen. Virol. 66, 2271–2277.

Chomzynsky, P. and Sacchi, N. (1987) Single step method of isolation of acid guanidine thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162, 156–159.

Chui, L.W., Vainionpaa, R., Marusyk, R., Salmi, A. and Norrby, E. (1986) Nuclear accumulation of measles virus nucleoprotein associated with a temperature sensitive mutant. J. Gen. Virol. 67, 2153–2161.

Cooper, P.D. (1964) The mutation of poliovirus by 5-fluorouracil. Virology 22, 186–192.

Cosby, S.L., Lyon, C., Rima, B.K. and Martin, S.J. (1985) The generation of small plaque mutants during undiluted passage of canine distemper virus. Intervirology 23, 157–166.

Danenberg, P.V. (1977) Thymidylate synthetase—a target enzyme in cancer chemotherapy. Biochim. Biophys. Acta 17, 4018–4024.

Danenberg, P.V. and Danenberg, K.D. (1978) Effect of 5,10-methylene tetrahydrofolate on the dissociation of 5-fluoro-2′-deoxyuridylate from thymidylate synthetase. Evidence for an ordered mechanism. Biochemistry 17, 4018–4024.

Danenberg, P.V. and Lockshin, A. (1982) Thymidylate synthetase substrate complex formation. Mol. Cell. Biochem. 43, 49–57.

Davern, C.I. and Bonner, J. (1958) The influence of 5-fluorouracil on Tobacco Mosaic virus production in tobacco leaf discs. Biochim. Biophys. Acta 29, 205–206.

De, B.P., Lesoon, A. and Banerjee, A.K. (1991) Human parainfluenza virus type 3 transcription in vitro: role of cellular actin in mRNA synthesis. J. Virol. 65, 3268–3275.

De Clercq, I.E., Descamps, J., Verhelst, G., Walker, R.T., Jones, A.S., Torrence, P.F. and Shergar, D. (1980) Comparative efficacy of antiherpes drugs against different strains of herpes simplex virus. J. Infect. Dis. 141, 563–574.

Dragun, M., Rada, B., Norotny, H. and Beranch, J. (1990) Antiviral activities of pyrimidine nucleoside analogues: some structure–activity relationships. Acta. Virol. Praha. 34, 321–329.

Feinberg, A.P. and Vogelstein, B. (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132, 6–13.

Field, A.K., Davis, M.E., DeWitt, C.M., et al. (1986) Efficacy of 2′-non-cyclic GMP in treatment of experimental herpes virus infections. Antiviral Res. 6, 329–341.

Fort, P.H., Marty, L., Piechaczyk, M., et al. (1985) Various rat adult tissue express only one major mRNA species from the glyceraldehyde 3-phosphate dehydrogenase multigene family. Nucl. Acids Res. 13, 1431–1442.

Galinski, M.S. and Wechesler, S.L. (1991) The molecular biology of the paramyxovirus genus. In: D.W. Kingsbury (Ed), The Paramyxoviruses, pp. 66–68. Plenum Press, New York.

Ghosh, A., Nayak, R. and Shaila, M.S. (1994) Altered ultrastructre of rinderpest virus and its nucleocapsids induced by 5-fluorouracil. Curr. Sci. 67, 441–446.

Ghosh, A., Joshi, V.D. and Shaila, M.S. (1995) Characterization of an in vitro transcription system from rinderpest virus. Vet. Microbiol. 44, 165–174.

Gordon, M.P. and Staehelin, M. (1959) Studies on the incorporation of 5-fluorouracil into a virus nucleic acid. Biochim. Biophys. Acta 36, 351–361.

Herrick, D. and Kufe, D.W. (1984) Lethality associated with incorporation of 5-fluorouracil into preribosomal RNA. Mol. Pharmacol. 26, 135–140.

Hu, J.M. and Hsiung, G.D. (1989) Evaluation of new antiviral agents. I. In vitro perspectives. Antiviral Res. 11, 217–232.

Hubbard, K.J., Baskerville, A. and Stephenson, J.R. (1991) Ability of mutagenized virus variant to protect young lambs from Rift valley fever. Am. J. Vet. Res. 52, 50–55.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurement with the folin-phenol reagent. J. Biol. Chem. 193, 265–275.

Madan Kumar, A. and Nayak, R. (1990) Glycosidic bond cleavage of 5-fluoro-2′-deoxyuridine and 5-fluorouridine by amino acyl-tRNA synthetases. Biochem. Biophys. Res. Commun. 173, 731–735.

Melnick, J.L. and Rapp, F. (1965) Use of antiviral compounds in analyzing the sequential steps in the replication of SV40 papovavirus. J. Exp. Med. 119, 313.

Moyer, S.A. and Horikami, S.N. (1991) The role of viral and host cell proteins in paramyxovirus transcription and replication. In: D.W. Kingsbury (Ed), The Paramyxoviruses, pp. 249–274. Plenum Press, New York.

Moyer, S.A., Baker, S.C. and Horikami, S.M. (1990) Host cell proteins required for measles virus reproduction. J. Gen. Virol. 71, 775–783.

Munyon, W. and Salzman, N.P. (1962) The incorporation of 5-fluorouracil into poliovirus. Virology 18, 95–101.

Reed, L.H. and Muench, H. (1938) A simple method of estimating 50% end points. Am. J. Hyg. 37, 493.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) In: Molecular Cloning—A Laboratory Manual, Vol. I, pp. 7.43–7.51. Cold Spring Harbor Laboratory Press, New York.

Shiba, T. and Saigo, K. (1982) Packaging of tRNA and 6S stable RNA into bacteriophage MS2 particles produced in *Escherichia coli* treated with 5-fluorouracil. Virology 119, 209–213.

Shimura, Y. and Nathans, D. (1964) Preparation of coliphage MS2 containing 5-fluorouracil. Biochem. Biophys. Res. Commun. 16, 116–120.

Spiegelman, S., Sawyer, R., Nayak, R., Ritzi, E., Stolfi, R. and Martin, D. (1980) Improving antitumor activity of 5-fluorouracil by increasing its incorporation into RNA via metabolic modulation. Proc. Natl. Acad. Sci. U.S.A. 77, 4966–4970.

Takehara, K., Min, M.K., Battles, J.K., et al. (1989) Identification of mutations in the mRNA of a candidate vaccine strain of Rift valley fever virus. Virology 169, 452–457.

Tolson, N.D., Charlton, K.M., Stewart, R.B., et al. (1990) Mutants of rabies virus in skunks: immune response and pathogenicity. Can. J. Vet. Res. 54, 178–183.

Vulimiri, S.V. and Nayak, R. (1993) 5-fluorouracil modulates aminoacylation of rat liver tRNA. Biochem. Mol. Biol. Int. 31, 1121–1128.

Vulimiri, S.V. and Nayak, R. (1994) 5-fluorouracil induces structural changes in rat liver tRNA. Biochem. Mol. Biol. Int. 33, 147–154.

Will, C.L. and Dolnick, B.J. (1989) 5-fluorouracil inhibits dihydrofolate reductase precursor mRNA processing and/or nuclear mRNA stability in methotrexate-resistant KB cells. J. Biol. Chem. 264, 21413–21421.

Wu, X-P. and Dolnick, B.J. (1993) 5-fluorouracil alters dihydrofolate reductase pre-mRNA splicing as determined by quantitative polymerase chain reaction. Mol. Pharmacol. 44, 22–29.

Yang, Z.H., Tolman, R.L., Colonno, R.J. and Hsiung, G.D. (1988) Antiviral effect of compound 2′-nor-CGMP against cytomegalovirus infection in guinea pig model. (Abstract). Antiviral Res. 9, 141.