ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1986, p. 1010–1016
0066-4804/86/061010-07$02.00/0

Vol. 29, No. 6

Effect of Ribavirin on Macromolecular Synthesis in Vesicular Stomatitis Virus-Infected Cells

PHILIP TOLTZIS[1]* AND ALICE S. HUANG[1,2]

Division of Infectious Diseases, Children's Hospital,[1] and Department of Microbiology and Molecular Genetics, Harvard Medical School,[2] Boston, Massachusetts 02115

Received 6 September 1985/Accepted 26 March 1986

Ribavirin at 200 μg/ml inhibited vesicular stomatitis virus (VSV) growth in Chinese hamster ovary (CHO) cells by 2.5 logs. To determine the mechanism of this inhibition, viral macromolecular synthesis was examined. VSV primary transcription remained unaffected, but overall VSV RNA synthesis decreased by 40 to 60%. When ribavirin was added 1.5 h after infection, inhibition of progeny production was partially lost, indicating that the antiviral effect was on an early stage after primary transcription. Inhibition of RNA polymerization by premature chain termination was not evident. Viral translation, on the other hand, was reduced by 95% with an inhibition of every protein species. Furthermore, viral RNA synthesized in the presence of ribavirin did not translate well in an in vitro translation system. In contrast, uninfected CHO cells treated with ribavirin showed a greater sensitivity in RNA synthesis than in protein synthesis. This suggests that the cellular translational machinery was not directly affected. Short-term treatment of cells resulted in negligible toxicity, but after 24 h there was marked alteration of cellular integrity. These results, taken together with data on other viruses, suggest that in the presence of ribavirin, dysfunctional VSV mRNA was synthesized, resulting in its failure to be translated. The selective antiviral effects of ribavirin and its relative lack of toxicity for host cells may be predicted on the basis of mRNA turnover and the requirements for de novo functional mRNA.

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a synthetic nucleoside that has broad spectrum antiviral activity in cell culture systems (22) and against many viral infections of laboratory animals. These observations, as well as the drug's low toxicity, have led to human clinical trials against diseases caused by influenza virus, hepatitis A and B viruses, herpesviruses, respiratory syncytial virus, Lassa fever virus, and human T-cell lymphotropic virus type III/lymphadenopathy-associated virus; its effectiveness in human disease is currently under evaluation (5, 15, 18, 23).

Ribavirin closely resembles guanosine in structure (21), and many of the observed in vitro effects of the drug can be reversed by the addition of guanosine (9, 11, 13, 16, 26). Ribavirin monophosphate inhibits cellular 5'-IMP dehydrogenase (IMPDH) (26). However, the molecular basis of its apparent selective antiviral effect remains elusive. Any unifying hypothesis regarding the mechanism of action of the drug is confounded by the possibility that its primary molecular target may be different for different viruses (23). Indeed, even the same virus may show variable sensitivity to ribavirin, depending upon host cell type as well as other culture conditions (14), suggesting a contribution of nonviral factors to its antiviral effect as well. To define more clearly the mechanism for the selective antiviral effects of ribavirin, the vesicular stomatitis virus (VSV) system was used with the hope that the molecular basis of the action of this drug could be determined with this well-defined virus. Our data suggest that ribavirin failed to inhibit the VSV polymerase selectively; rather, in the presence of drug, viral primary transcription resulted in the synthesis of poorly translated mRNAs, leading to reduced viral protein synthesis and subsequent inhibition of genomic replication and secondary transcription.

MATERIALS AND METHODS

Virus cells and materials. Chinese hamster ovary (CHO) cells were grown at 37°C in Joklik modified minimal essential medium enriched with 2% fetal calf serum and 1% nonessential amino acids. VSV (Indiana serotype, San Juan strain) was obtained from sucrose gradient purified laboratory stock and inoculated at a multiplicity of 20 in all virus experiments. [^{14}C]uridine (>50 mCi/mmol) and [^{35}S]methionine (>400 Ci/mmol) were both obtained from New England Nuclear Corp. (Boston, Mass.). Ribavirin was obtained from Viratek (Covina, Calif.). Ribonuclease A was obtained from Calbiochem-Behring (La Jolla, Calif.) and ribonuclease T_1 from Bethesda Research Laboratories, Inc. (Gaithersburg, Md.). Actinomycin D was a kind gift from Merck Sharp and Dohme (Rahway, N.J.). The rabbit reticulocyte lysate translation kit was purchased from New England Nuclear.

Sensitivity testing for growth inhibition of VSV. Monolayers of CHO cells grown in petri dishes (60 by 15 mm) were infected with VSV suspended in a 0.2-ml volume. After a 0.5-h attachment period, the cells were washed and then incubated with 5 ml of medium at 37°C in a 5% CO_2 incubator. Ribavirin at different concentrations was added to the medium at the indicated times, and at given intervals small samples of medium were removed for plaque assay.

Radiolabeled precursor studies. Radiolabeled incorporation studies were performed by growing parallel infected and uninfected 2-ml suspension cultures of CHO cells at a concentration of 2.0×10^6 cells per ml with increasing concentrations of ribavirin. Details of these pilot assays have been previously published (7). With [^{35}S]methionine incorporation the experiments were performed in the absence of

* Corresponding author.

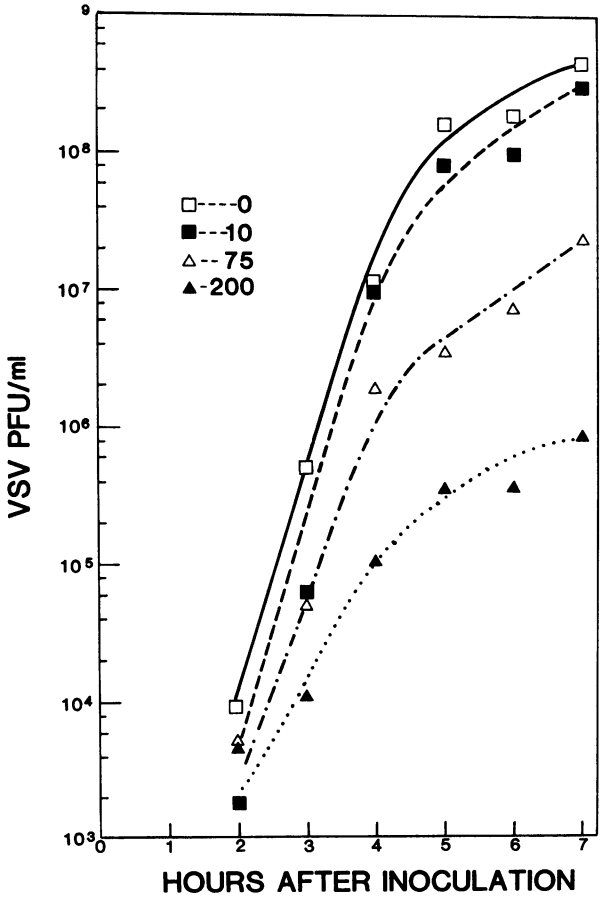

FIG. 1. Degree of antiviral effect of ribavirin on VSV growth. Monolayers of CHO cells were infected with VSV in minimal essential medium containing 0, 10, 75, and 200 μg of ribavirin per ml. Samples were withdrawn hourly and assayed by plaque formation on CHO cells.

actinomycin D, whereas with [^{14}C]uridine, actinomycin D at 5 μg/ml was present.

RESULTS

Inhibition of VSV by ribavirin. The sensitivity of VSV to ribavirin was assayed by measuring the growth of infectious progeny in the presence of increasing concentrations of drug. When ribavirin at 200 μg/ml was added immediately after viral attachment, the production of infectious virus was suppressed by approximately 2.5 logs (Fig. 1). At lower concentrations, the antiviral effects were considerably more moderate. Moreover, the time at which the drug was added to the infected cells substantially affected its antiviral capabilities (Table 1). Delaying the addition of ribavirin for 1 h after attachment of virus led to virtually no antiviral effect of the drug when it was added at a concentration of 50 μg/ml and reduced a substantial portion of the effect when it was added at 200 μg/ml. Further delaying the addition of ribavirin led to a greater reduction of antiviral activity.

Effect of ribavirin on [^{14}C]uridine incorporation into RNA by uninfected and VSV-infected cells. Because the prior experiment had suggested that ribavirin exerted its effect at an early event in viral metabolism, the effect of ribavirin on viral RNA synthesis over a 3.5-h period was tested (Fig. 2). Surprisingly, ribavirin led to only a relatively mild depression of synthesis of virus-specific RNA; even at 200 μg/ml, at

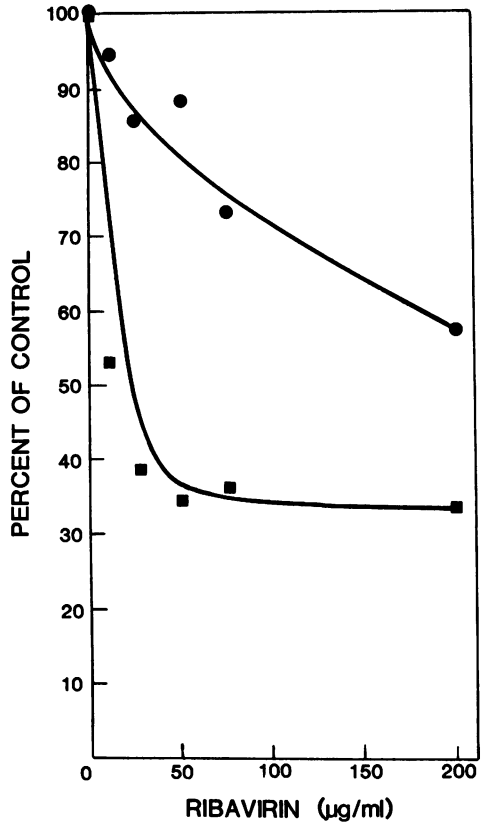

FIG. 2. Effect of ribavirin on viral and cellular RNA synthesis. VSV was grown in suspension cultures in the presence of actinomycin D (5 μg/ml) with increasing concentrations of ribavirin added at time of inoculation. At 0.5 h after infection [^{14}C]uridine was added. At 4 h after infection samples were precipitated in cold trichloroacetic acid, filtered, and counted. Parallel cultures of mock-infected cells were grown (without actinomycin) and handled identically. Results are graphed as percentage of counts compared with control (grown in the absence of ribavirin) versus concentration of drug. Symbols: ●, virus-infected cells; ■, mock-infected cells.

TABLE 1. Loss of antiviral activity by delayed addition of ribavirin to infected cells[a]

Time of addition (h postinfection)	Ribavirin (μg/ml)	Virus yield (PFU/ml)
0	0	2.7×10^7
0.5	50	5.1×10^6
	200	1.2×10^5
1.5	50	2.0×10^7
	200	5.0×10^6
2.5	50	1.4×10^7
	200	9.0×10^6
3.5	50	2.0×10^7
	200	1.7×10^7

[a] CHO cells were infected with VSV as described in the legend to Fig. 1, except that ribavirin was added at the indicated times after infection. At 3.5 h all monolayers were washed with phosphate-buffered saline, and the overlay containing ribavirin at the same concentration as the prewash exposure was added again. At 7 h after inoculation the titer of virus in each overlay was determined by plaque assay.

TABLE 2. Effect of actinomycin D on ribavirin inhibition of VSV growth in CHO cells[a]

Treatment of cells with drug (μg/ml):		Virus yield (PFU/ml)
Ribavirin	Actinomycin D	
0	0	1.0×10^7
50	0	1.8×10^6
50	5	1.1×10^7
200	0	1.0×10^5
200	5	4.7×10^6

[a] Viral growth was assayed in a manner identical to that described in the legend to Fig. 1. After a 0.5-h attachment period ribavirin was added with and without actinomycin D at the concentrations listed.

which level production of infectious virus was suppressed by greater than two logs, viral RNA production was approximately suppressed by only 40%. Furthermore, the effect of ribavirin on cellular RNA synthesis was found to be more marked than on viral RNA production. Labeling infected and uninfected cells for a shorter period, 2.5 h, resulted in similar results (data not shown).

Effect of actinomycin D on the viral inhibition exerted by ribavirin. A possible artifact that may have affected the incorporation of [^{14}C]uridine into VSV macromolecules was that resulting from the presence of actinomycin D. By inhibiting cellular RNA synthesis, actinomycin D may have increased the cellular GTP pools, which in turn may have led to a reversal of the drug's antiviral effect by competition at its molecular target (13, 14). The possibility that actinomycin D resulted in an underestimation of the inhibition of viral RNA synthesis was approached indirectly by first determining whether actinomycin D influenced the drug's effect on viral progeny production. In fact, the addition of actinomycin D to ribavirin-treated, VSV-infected cells entirely reversed the inhibition at 50 μg of ribavirin per ml and decreased the inhibition at 200 μg/ml (Table 2). These results indicate that the antiviral activity of ribavirin on VSV may indeed be significantly influenced by the presence of actinomycin D.

Determination of the absolute amounts of viral RNA present in ribavirin-treated cells. Though actinomycin D substantially reduced the antiviral activity of ribavirin as measured by the production of infectious progeny, it nevertheless remained undetermined whether this reduction was due to an effect at the level of viral transcription. Indeed, other investigators have questioned the correlation between effect of ribavirin upon cellular GTP stores and its effect upon viral transcription (27, 28). Therefore, these results on infectivity indicated the need to confirm the inhibition measured by uridine incorporation in virus-infected cells by direct assay requiring no actinomycin D. This was done by hybridizing excess radioactive VSV genomic RNA to total cellular RNA produced in the presence of ribavirin (Table 3). The suppression of synthesis of hybridizable viral RNA measured by this direct assay is slightly greater than had been noted in the studies involving [^{14}C]uridine incorporation in the presence of actinomycin D. However, the degree of suppression was still far less than the suppression of progeny virions. Furthermore, this experiment indicated that the addition of actinomycin D to ribavirin-treated infected cells had little effect on the degree of inhibition of total viral RNA synthesis.

TABLE 3. Viral RNA produced in the presence of ribavirin[a]

Ribavirin (μg/ml)	RNase-resistant RNA (cpm)	% Synthesis[b]
0	2,101 and 2,003	100
25	1,485 and 1,535	74
50	1,168 and 1,263	59
200	850 and 1,068	47

[a] Total cellular RNA was extracted at 4 h after infection from 1.0×10^5 VSV-infected CHO cells treated with 0, 50, and 200 μg of ribavirin per ml by phenol extraction and ethanol precipitation (21). VSV genomic RNA was extracted from sucrose gradient-purified, [^{3}H]-uridine-labeled VSV as previously described (2). Radiolabeled RNA was added in excess (approximately 0.5 μg) to each sample and hybridized for 2 h at 70°C as detailed by Clinton et al. (2). RNase-resistant fractions were determined by precipitation in cold trichloroacetic acid. The counts per minute of duplicate samples are given.

[b] Expressed as percentage of control using the average value of duplicate samples. Probe self annealed at 2%.

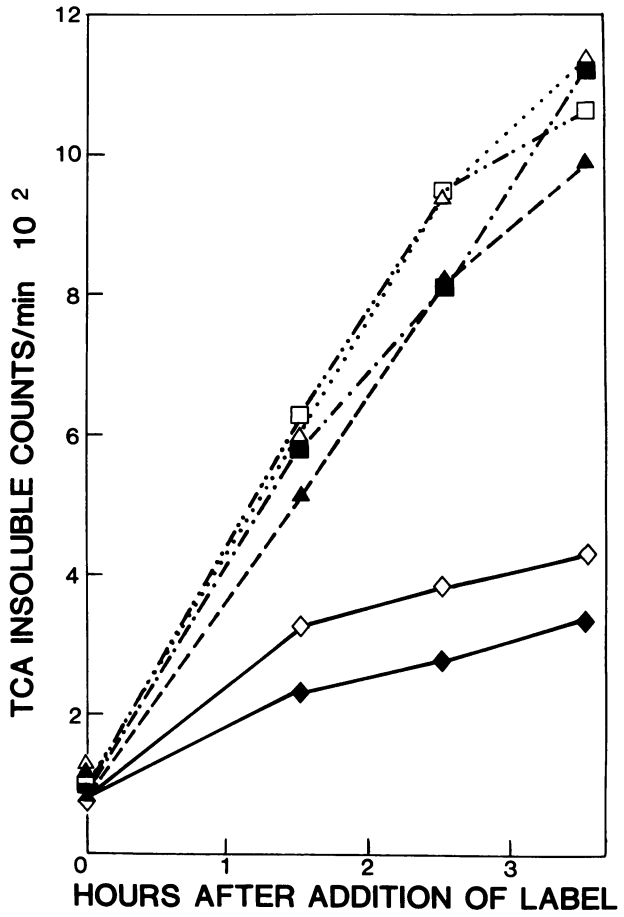

FIG. 3. Effect of ribavirin on viral primary transcription. Growing cells were incubated in suspension cultures containing actinomycin D (5 μg/ml), cycloheximide (50 μg/ml), and increasing concentrations of ribavirin. At 0.5 h after infection, [^{14}C]uridine was added. At 1.5, 2.5, and 3.5 h after addition of radioactive label samples were removed and precipitated in trichloroacetic acid (TCA). Symbols: □, no ribavirin added; ■, 50 μg of ribavirin per ml; △, 125 μg of ribavirin per ml; ▲, 200 μg of ribavirin per ml; ◇, mock-infected cells plus actinomycin D and cycloheximide; ◆, mock-infected cells plus actinomycin D, cycloheximide, and 200 μg of ribavirin per ml.

Effect of ribavirin on viral primary transcription. To examine further whether inhibition of VSV RNA synthesis was primarily directed against transcription or replication of the genome, the effect of ribavirin on VSV primary transcription was assessed. Incorporation of radioactive uridine into vi-

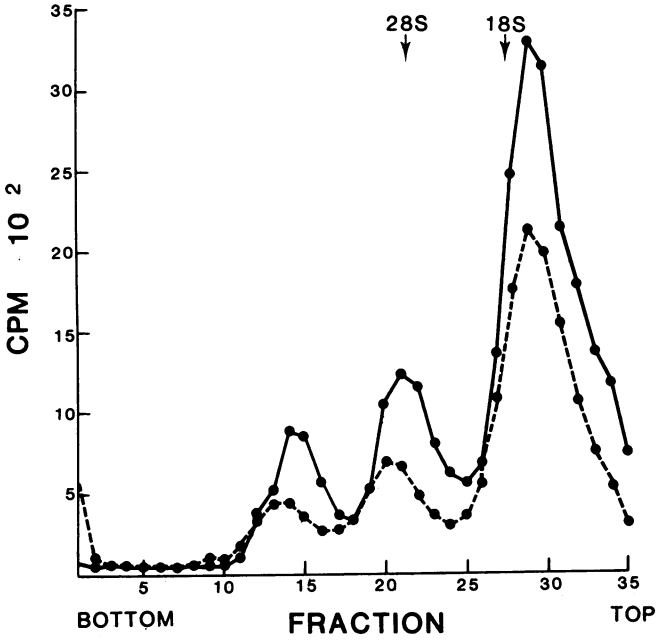

FIG. 4. Separation of VSV RNAs synthesized in the presence of ribavirin. VSV was grown in suspension cultures in the presence of 5 μg of actinomycin D per ml with and without ribavirin (200 μg/ml). [^{14}C]uridine was added 0.5 h after infection. At 4 h after infection, the cells were harvested and lysed in hypotonic buffer containing 1% Nonidet P-40. The resultant cytoplasmic extract was layered onto a 15 to 30% sodium dodecyl sulfate-sucrose gradient and centrifuged by the method of Stampfer et al. (25). Fractions (1 ml) were precipitated in cold trichloroacetic acid, filtered, and counted. RNAs (28S and 18S) from uninfected cells centrifuged in parallel gradients were detected by optical density and used as markers. ●——●, Without ribavirin; ●- - -●, with ribavirin.

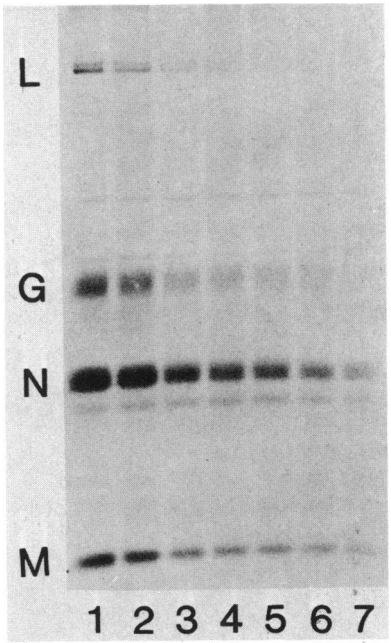

FIG. 5. Effect on ribavirin on viral protein synthesis. VSV was grown in suspension cultures of CHO cells with increasing concentrations of ribavirin. At 2.5 h after infection [^{35}S]methionine was added. At 4 h the cells were harvested and lysed in hypotonic buffer containing 1% Nonidet P-40, electrophoresed on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiographed. L, G, N, and M represent viral proteins. Lanes 1 through 7 show samples grown in 0, 3, 6, 10, 25, 50, and 200 μg of ribavirin per ml, respectively.

rus-infected cells in the presence of actinomycin D and cycloheximide showed no significant differences in the rate of primary transcription whether ribavirin was present or not (Fig. 3). This experiment suggests that ribavirin fails to have any effects on the VSV polymerase.

Effect of ribavirin on viral RNA replication versus transcription. The nature of the suppression of viral RNA synthesis by ribavirin was further examined by separating radioactively labeled viral genomic and mRNAs made in the presence of the drug, using rate-zonal centrifugation. The degree of inhibition, about 50%, of the 28S and 13S-18S viral mRNAs resulting from ribavirin treatment was identical to that of 40S genomic RNA synthesis (Fig. 4). These data added further support to the theory that the inhibition of total VSV RNA synthesis produced by ribavirin was a result of inhibition of viral RNA replication and subsequent secondary transcription rather than primary transcription. In addition, the absence of significant amounts of radioactivity skewed towards the top of the gradient demonstrated that ribavirin treatment did not result in the production of short pieces of viral RNA because of premature termination.

Effect of ribavirin on cellular and viral protein synthesis. To assess the effect of ribavirin on the translation of viral mRNA, infected cells treated with drug were labeled with [^{35}S]methionine, and the viral proteins were separated on polyacrylamide gels (Fig. 5). Quantification of the individual viral proteins established that no individual protein was suppressed disproportionately at any given concentration of ribavirin. To compare the amount of total viral proteins to the amount of cellular proteins produced in the presence of ribavirin, viral proteins were quantitated by densitometry and compared with uninfected cells (Fig. 6). The degree of suppression of viral protein synthesis was greater than 90% at 200 μg/ml, which was disproportionately greater than that of total viral RNA synthesis and approached the degree of suppression seen for the production of infectious virus, suggesting that VSV RNA being transcribed was poorly translated. In contrast, protein synthesis in uninfected CHO cells was only mildly altered, with approximately 30% inhibition even at a concentration of 200 μg of ribavirin per ml. The effects of this drug on viral and cellular macromolecular synthesis were therefore quite different. Ribavirin suppressed VSV RNA synthesis to a mild degree but had a more dramatic effect on viral protein synthesis; by contrast, the drug suppressed cellular RNA synthesis to a greater degree than synthesis of cellular protein.

In vitro translation of VSV mRNAs synthesized in the presence of ribavirin. To confirm the conclusions drawn from the above data that ribavirin led to the production of poorly-translated VSV mRNAs, viral messages made in the presence (200 μg/ml) and absence of drug were isolated and translated in an in vitro rabbit reticulocyte system. Messenger RNA was synthesized in the absence of actinomycin D. After purification, the relative quantities of virus-specific messages in the ribavirin-treated and untreated samples were determined by quantitative RNA-RNA hybridization. These assays indicated that the ribavirin-treated samples contained approximately 40% of virus-specific mRNAs compared with control (data not shown), a result similar to that determined by the sucrose-gradient analysis of RNA species described above, in which actinomycin D was included. This

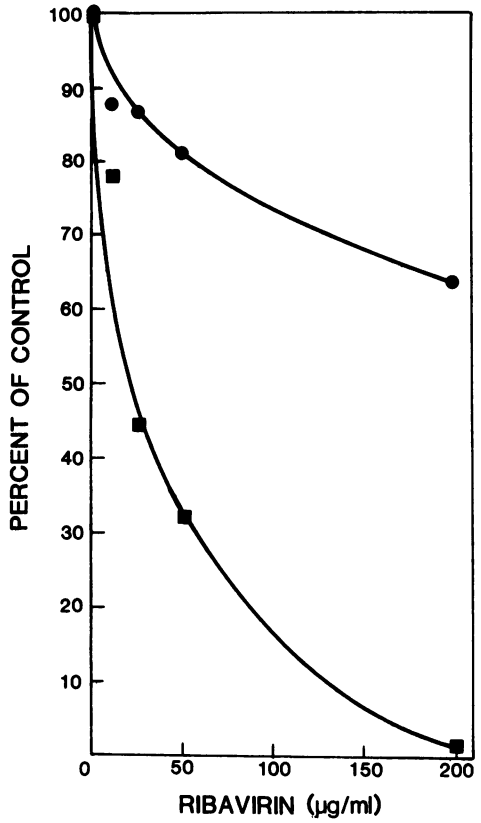

FIG. 6. Comparison of viral and cellular protein synthesis in the presence of ribavirin. The relative amounts of viral proteins electrophoresed in Fig. 5 were determined by densitometry and graphed as percentage of counts compared with control versus concentration of ribavirin. A series of mock-infected samples grown in parallel to the infected cells was labeled identically. After 4 h small samples of the uninfected cells were trichloroacetic acid precipitated, and the relative counts were graphed in a similar manner. Symbols: ■, virus-infected cells; ●, mock-infected cells.

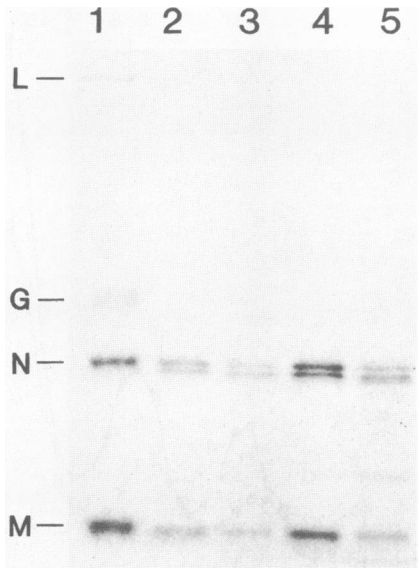

FIG. 7. In vitro translation of VSV mRNAs. CHO cells (8×10^7) were infected with VSV at high multiplicity in the presence (200 μg/ml) and absence of ribavirin. After 4 h the cells were harvested and lysed with Nonidet P-40, and the cytoplasmic extract was centrifuged through a 20 to 40% CsCl gradient at 55,000 × *g* for 16 h. The pellet was collected and precipitated in ethanol, and messenger RNA was selected by oligo(dT)-cellulose chromatography with standard methods. After purification, relative amounts of virus-specific RNA were assayed by methods described in Table 3, footnote *a*. Equal quantities of VSV mRNA (approximately 75 ng) were added to a commercial rabbit reticulocyte in vitro translation system by using the reaction mixture suggested by the manufacturer (80 mM potassium acetate, 1 mM magnesium acetate) under conditions of ribosome excess. Proteins were labeled with [^{35}S]methionine. Lanes: 1, VSV protein standards; 2, control RNA, 45-min reaction; 3, ribavirin-treated RNA, 45-min reaction; 4, control RNA, 90-min reaction; 5, ribavirin-treated RNA, 90-min reaction. L, G, N, and M represent viral proteins.

confirmed that the addition of actinomycin D had little influence upon viral transcription in the presence of ribavirin in this system.

When equal amounts of ribavirin-treated and control mRNAs were translated in vitro, substantially less protein was synthesized from the ribavirin-treated samples (Fig. 7). Differences in protein production were quantified by cutting out the bands representing virus-specific protein and measuring the radioactivity directly. After a 90-min translation reaction, M protein synthesis was reduced by approximately 40% (16,681 cpm versus 28,029 cpm), and N protein synthesis was reduced by 75% (16,079 cpm versus 69,760 cpm) in the ribavirin-treated samples. These data confirmed that the mRNAs synthesized in the presence of ribavirin were poorly translated.

Effect of ribavirin on cellular integrity. The consequence of the effects of ribavirin on the viability of CHO cells was assessed by cell division and ability to exclude vital dye (Table 4). For up to 5 h of treatment with ribavirin, within the time frame in which the above experiments were conducted, there was no discernable effect on viability. However, after 24 h, considerable cellular toxicity, as well as inhibition of division, was noted. Therefore, in the short term, when viral replication was ongoing, ribavirin had little effect on cell survival, but over a longer period the effects of ribavirin on host macromolecular synthesis markedly compromised cellular integrity.

DISCUSSION

We used the VSV system to help define the molecular activity of ribavirin. Though VSV is not as sensitive as other viruses to this drug, it is generally listed among the viruses inhibited by ribavirin (23). It has also met the major requirement for use as a model system: namely, that antiviral effects can be distinguished from cellular effects. In addition, its ability to grow rapidly to high titer and the relative technical ease with which its molecular biology can be probed make VSV a productive model system with which to work.

Past reports have suggested three different, though not mutually exclusive, modes of action for ribavirin. The first suggests that ribavirin suppresses cellular de novo production of GMP by inhibiting IMPDH; the resultant depletion of GTP serves to starve the virus of the substrate for its own transcription (1, 14, 26). Suppression of IMPDH by ribavirin 5′-monophosphate has been noted in several cell lines and activity against this enzyme in CHO cells has been demonstrated for an analog of this compound (10). However, objections have been raised to this hypothesis, most notably that derivatives of ribavirin have been developed in which the activities against viruses and IMPDH can be separated

TABLE 4. Effect of ribavirin on CHO cell division and viability[a]

Time sampled after ribavirin (h)	Ribavirin (μg/ml)	10^4 Cells/ml	% Dead cells
0	0,3,6,75 and 200	26–28	≤5
45	0	30	≤5
	3	43	≤5
	6	38	≤5
	75	38	≤5
	200	33	≤5
24	0	87	1
	3	64	1
	6	40	3
	75	27	24
	200	21	36

[a] Cells were grown in a 50-ml suspension culture in the presence of ribavirin at concentrations indicated. At 5 and 24 h after exposure to drug, cells were counted in a hemacytometer, and their viability was assessed by trypan blue exclusion as judged by examination through a light microscope.

(24). Furthermore, it has been questioned whether the reduction of cellular GTP pools noted after ribavirin treatment is sufficient to inhibit viral transcription (27). A second theory proposes that ribavirin acts by suppressing virus-specific polymerases. This has been reported for influenza virus in tests involving ribavirin triphosphate in a cell-free polymerase assay (3, 28, 29). However, some data report only mild suppression of VSV polymerase activity (3), and other data indicate no inhibition toward a wide variety of animal and bacterial RNA polymerases (24). A final hypothesis states that ribavirin acts by inhibiting the formation of the 5′ cap structure on messenger RNA. This theory is supported by reports indicating an inhibition of vaccinia guanylyl transferase in the presence of ribavirin triphosphate (4) and the absence of cap structures on RNA isolated from cells infected with Venezuelan equine encephalitis virus (P. G. Canonico, J. S. Little, P. B. Jahrling, and E. L. Stephen, Program Abstr. 19th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 977, 1979). In addition, resistance to ribavirin has been found with some of the picornaviruses (24) which do not contain a cap structure on their RNA (6, 17). This resistance is not seen with all picornaviruses; however, single cycle metabolic studies similar to those presented for VSV are lacking.

The data presented on VSV support the hypothesis that ribavirin exerts its antiviral effect primarily by production of poorly translated mRNA. This hypothesis was deduced from data examining intracellular events and confirmed in a cell-free, in vitro translation system. Because synthesis of viral primary RNA transcripts was uninhibited in the presence of ribavirin, it was likely that the overall suppression by ribavirin on total VSV RNA synthesis actually represented an indirect effect of the drug. VSV requires continuous synthesis of proteins to replicate its genomic RNA (19); interference with viral translation will lead to decreased viral replication and subsequent secondary transcription.

The basis for the dysfunction of viral mRNA made in the presence of ribavirin remains undetermined. However, several of our experiments provide preliminary data which exclude possible mechanisms. The reduction of the antiviral activity of ribavirin when the drug was added only 1 h after attachment supports the concept that functional mRNA made at an early stage of the viral growth cycle in the absence of drug was enough to lead to normal progeny production. Therefore, whether or not ribavirin was incorporated into mRNA or genomic RNA, it had no apparent mutagenic effect on infectious progeny. Indeed, others have found little incorporation of ribavirin into RNA (24). Furthermore, VSV mRNA made in the presence of ribavirin and sized by gradient centrifugation was full-length, rendering premature chain termination an unlikely basis for mRNA dysfunction. Finally, the differential effect of ribavirin on cellular and VSV protein synthesis indicates that there is no primary toxicity toward cellular protein synthetic machinery. Taken together, these data indicate that full-length viral mRNA is synthesized but poorly translated in the absence of any direct effect on cellular protein synthesis. Therefore, though the basis for the dysfunctional translation of these mRNAs was not directly identified by these data, exclusion of these other possibilities may suggest interference with 5′ cap formation.

Actinomycin D was noted to reverse the antiviral activity of ribavirin on VSV in these studies. The same reversal was noted in Sindbis virus infections as well (12). These observations underline the caution that must be exercised when interpreting studies involving the effect of ribavirin in which actinomycin has been used. However, in our studies on VSV, the inhibition of ribavirin on RNA synthesis was internally consistent and was supported by experiments utilizing no actinomycin. Furthermore, when actinomycin D was present the degree of inhibition detected was only slightly lower than when actinomycin was absent. Therefore, though actinomycin D had significant effects on the antiviral activity of ribavirin, little effect was noted upon viral transcription. These observations allowed the use of actinomycin D when assessing transcriptional activity in the presence of drug. They also added further support to the hypothesis that the major molecular target of ribavirin was not at the level of transcription in this system. The basis for the reversal of the antiviral activity of ribavirin by actinomycin D has been attributed to an increase in cellular GTP pools resulting from actinomycin treatment (12). However, the observation that actinomycin D may suppress expression of interferon and other cellular gene products normally induced by VSV (30) suggests this reversal may be complex and multifactorial.

Ribavirin's effects on uninfected CHO cell viability were in disagreement with prior results which showed that exposure to ribavirin inhibits cellular proliferation but generally does not kill the cells at concentrations less than 100 μg/ml (8). In the system examined in this report, some inhibition of growth was seen at 3 μg/ml, almost total inhibition of cell division was noted by 6 μg/ml, and substantial toxicity was found at 75 μg/ml. Our CHO cells were grown exponentially, a condition that presumably requires high turnover of cellular macromolecules, which may account for the relatively greater degree of toxicity we observed. Over the short term, cellular integrity was maintained, presumably by translation of stable mRNAs synthesized before ribavirin exposure.

ACKNOWLEDGMENTS

This work was supported by Public Health Services grant AI 16625/20896 from the National Institutes of Health. P.T. was supported by the Institutional National Research Service award T32 CA 09031.

We thank Trudy Lanman for laboratory support and Kenneth McIntosh and Jean Patterson for their helpful suggestions. We also thank Barbara Connolly for computer text editing of the manuscript.

LITERATURE CITED

1. **Browne, M. J.** 1978. Mechanism and specificity of action of ribavirin. Antimicrob. Agents. Chemother. **15**:747–753.

2. **Clinton, G. M., S. P. Little, E. S. Hagen, and A. S. Huang.** 1978. The matrix (M) protein of vesicular stomatitis virus regulates transcription. Cell **15**:1455–1462.
3. **Eriksson, B., E. Helgstrand, N. G. Johannson, A. Larsson, A. Misiorny, J. O. Noren, L. Philipson, K. Stenberg, G. Stening, S. Stridh, and B. Oberg.** 1977. Inhibition of influenza virus ribonucleic acid polymerase by ribavirin triphosphate. Antimicrob. Agents Chemother. **11**:946–951.
4. **Goswami, B. B., E. Borek, D. K. Sharma, J. Fujitaki, and R. A. Smith.** 1979. The broad spectrum antiviral agent ribavirin inhibits capping of mRNA. Biochem. Biophys. Res. Commun. **89**:830–836.
5. **Hall, C. B., J. B. McBride, E. E. Walsh, D. M. Bell, C. L. Gala, S. Hildreth, L. G. Ten Eyck, and W. J. Hall.** 1983. Aerosolized ribavirin treatment of infants with respiratory syncytial virus infection. N. Eng. J. Med. **308**:1443–1447.
6. **Hewlett, M. J., J. K. Rose, and D. Baltimore.** 1976. The 5′ terminal structure of poliovirus polyribosomal RNA is pUUp. Proc. Natl. Acad. Sci. USA **73**:327–330.
7. **Huang, A. S., D. Baltimore, and M. Stampfer.** 1970. Ribonucleic acid synthesis of vesicular stomatitis virus. III. Multiple complementary messenger RNA molecules. Virology **42**:946–957.
8. **Huffman, J. N., R. W. Sidwell, G. P. Khare, J. T. Witkowski, L. B. Allen, and R. K. Robins.** 1973. In vitro effect of 1-β-D-ribofuranosyl-1, 2, 4-triazole-3-carboximide (virazole, ICN 1229) on deoxyribonucleic acid and ribonucleic acid viruses. Antimicrob. Agents Chemother. **3**:235–241.
9. **Knight, V., S. Z. Wilson, D. W. Alling, R. V. Moore, and R. M. Longoria.** 1981. Lack of interference of guanosine with ribavirin aerosol treatment of influenza A infection in mice. Antimicrob. Agents Chemother. **20**:477–480.
10. **Kuttan, R., R. K. Robins, and P. P. Saunders.** 1982. Inhibition of inosinate dehydrogenase by metabolites of 2-β-D-ribofuranosyl thiazole-4-carboxmide. Biochem. Biophys. Res. Commun. **107**:862–868.
11. **Larsson, A., K. Stenberg, and B. Oberg.** 1978. Reversible inhibition of cellular metabolism by ribavirin. Antimicrob. Agents Chemother. **13**:154–158.
12. **Malinoski, F., and V. Stollar.** 1980. Inhibition of Sindbis virus replication in *Aedes albopictus* cells by virazole (ribavirin) and its reversal by actinomycin: a correction. Virology **102**:473–476.
13. **Malinoski, F., and V. Stollar.** 1981. Inhibition of Sindbis virus replication by ribavirin: influence of cultural conditions and of the host cell phenotype. Antiviral Res. **1**:287–299.
14. **Malinoski, F., and V. Stollar.** 1981. Inhibitors of IMP dehydrogenase prevent Sindbis virus replication and reduce GTP levels in *Aedes albopictus* cells. Virology **110**:281–291.
15. **McClung, H. W., V. Knight, B. E. Gilbert, S. Z. Wilson, J. M. Quarles, and G. W. Divine.** 1983. Ribavirin aerosol treatment of influenza B virus infection. J. Am. Med. Assoc. **249**:2671–2674.
16. **Muller, W. E., A. Maidhof, H. Taschner, and R. K. Zahn.** 1977. Virazole (1-β-D-ribofuranosyl-1,2,4-trizole-3-carboximide): a cytostatic agent. Biochem. Pharmacol. **26**:1071–1075.
17. **Nomoto, A., Y. F. Lee, and E. Wimmer.** 1976. The 5′ end of polyvirus mRNA is not capped with $m^7G(5')$ ppp (5′)Np. Proc. Natl. Acad. Sci. USA **73**:375–380.
18. **Patki, S. A., and P. Gupta.** 1982. Evaluation of ribavirin in the treatment of acute hepatitis. Chemotherapy (Basel) **28**:298–303.
19. **Perlman, S. M., and A. S. Huang.** 1973. RNA synthesis of Vesicular Stomatis Virus. V. Interactions between transcription and replication. J. Virol. **12**:1395–1400.
20. **Prusiner, P., and M. Sundaralingam.** 1973. A new class of synthetic nucleoside analogues with broad-spectrum antiviral properties. Nature (London) New Biol. **244**:116–117.
21. **Rose, J. K., and D. Knipe.** 1975. Nucleotide sequence complexities, molecular weights, and poly(A) content of the vesicular stomatitis virus mRNA species. J. Virol. **15**:994–1003.
22. **Sidwell, R. W., J. N. Huffman, G. P. Khare, L. B. Allen, J. T. Witkowski, and R. K. Robins.** 1972. Broad spectrum antiviral activity of virazole: 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide. Science **177**:705–706.
23. **Sidwell, R. W., R. K. Robins, and I. W. Hillyard.** 1979. Ribavirin: an antiviral agent. Pharmacol. Ther. **6**:123–146.
24. **Smith, R. A.** 1980. Mechanism of action of ribavirin, p. 99 *In* R. A. Smith and W. Kirkpatrick (ed.), Ribavirin, a broad spectrum antiviral agent. Academic Press, New York.
25. **Stampfer, M., D. Baltimore, and A. S. Huang.** 1969. Ribonucleic acid synthesis of vesicular stomatitis virus. I. Species of ribonucleic acid found in Chinese hamster ovary cells infected with plaque-forming and defective particles. J. Virol. **4**:154–161.
26. **Streeter, D. G., J. T. Witkowski, G. P. Khare, R. W. Sidwell, R. J. Bauer, R. K. Robins, and L. N. Simon.** 1973. Mechanism of action of 1-β-ribofuranosyl-1,2,4-Triazole-3-carboxamide, a new broad spectrum antiviral agent. Proc. Natl. Acad. Sci. **70**:1174–1178.
27. **Stridh, S.** 1983. Determination of ribonucleoside triphosphate pools in influenza A virus-infected MDCK cells. Arch. Virol. **77**:223–229.
28. **Wray, S. K., B. F. Gilbert, and V. Knight.** 1985. Effect of ribavirin triphosphate on primed generation and elongation during virus transcription *in vitro*. Antiviral Res. **5**:39–48.
29. **Wray, S. K., B. F. Gilbert, M. W. Noall, and V. Knight.** 1985. Mode of action of ribavirin: effect of nucleotide pool alterations on influenza virus ribonucleoprotein synthesis. Antiviral Res. **5**:29–37.
30. **Zullo, J. W., B. H. Cochran, A. S. Huang, and C. D. Stiles.** 1985. Platelet-derived growth factor and double-stranded ribonucleic acids stimulate expression of the same genes in 3T3 cells. Cell **43**:793–800.