Inhibition of Rotaviruses by Selected Antiviral Substances: Mechanisms of Viral Inhibition and In Vivo Activity

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Several RNA virus inhibitors were evaluated against simian (SA11) rotavirus infections in vitro and murine rotavirus gastroenteritis in vivo. Test compounds included 1- β -D-ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin), 3-deazaguanine (3-DG), 3-deazauridine, and 9-(S)-(2,3-dihydroxypropyl)adenine [(S)-DHPA]. All drugs inhibited total infectious SA11 virus yields in MA-104 cells. Ribavirin, 3-DG, and (S)-DHPA affected [³H]uridine uptake into uninfected MA-104 cells in both the acid-soluble and -insoluble fractions. All drugs reduced the levels of dense (precursor) and light (complete) SA11 particle yields compared with controls but did not alter the relative amounts of dense compared with light particles, suggesting that the agents did not interfere with virus assembly. Ribavirin and 3-DG inhibited SA11 polypeptide synthesis, as determined by polyacrylamide gel electrophoresis studies. None of the agents or mono- and triphosphate derivatives of ribavirin inhibited SA11 RNA polymerase activity. In murine rotavirus studies, oral therapy with ribavirin-2',3',5'-triacetate and (S)-DHPA increased mean survival time, but no increase in survivor rate was observed. 3-DG- and (S)-DHPA-treated mice had a more rapid weight gain than controls, suggesting a probable lessening of the severity of the disease.

Reovirus-like agents (rotaviruses) have been associated with acute neonatal gastroenteritis in a number of animals, including humans (17, 26). Attempts to prevent the disease with vaccines have been encouraging but not highly successful to date (25, 30). Other methods have focused on treating the symptoms of the disease by parental replacement of fluids and electrolytes (13). Two experimental attempts have been made to treat the disease with antiviral agents. Schoub and Prozesky (22) demonstrated that 1- β -D-ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin) inhibited simian (SA11) rotavirus cytopathic effect in vitro but was ineffective against murine rotaviral disease in mice. In a study we have reported previously (24), the in vitro efficacies of ribavirin, 3-deazaguanine (3-DG), 3-deazauridine (3-DU), and 9-(S)-(2,3-dihydroxypropyl)adenine [(S)-DHPA] against bovine, porcine, and simian rotaviruses were determined. These results indicated 3-DG and ribavirin to be the most active rotaviral inhibitors of those examined; moderate antirotavirus activity was exhibited by (S)-DHPA and 3-DU. Of the three viruses evaluated, porcine rotavirus appeared to be least sensitive to the antiviral agents. The encouraging results of these studies led us to evaluate the compounds in more detail against the simian rotavirus in vitro to begin to

determine their mechanisms of rotavirus inhibition and to study the most likely candidate compounds against murine rotavirus in vivo. The results of these studies are presented in this report.

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MATERIALS AND METHODS

Antiviral compounds. Ribavirin, ribavirin 5'-monophosphate, ribavirin 5'-triphosphate, ribavirin triacetate, 3-DG, and 3-DU were obtained from ICN Pharmaceuticals, Covina, Calif. (S)-DHPA was provided by Eric DeClercq, Rega Institute of the Catholic University of Leuven, Leuven, Belgium. Each drug was dissolved in cell culture medium at an initial concentration of 2,000 μ g/ml for in vitro studies.

Viruses. The SA11 strain of simian rotavirus, obtained from Mary Estes, Baylor College of Medicine, Houston, Tex., and the murine rotavirus, obtained from Microbiological Associates, Bethesda, Md., were used. Pools of simian rotavirus were prepared in monkey kidney (MA-104) cells in the presence of 1 μ g of partially purified trypsin (ICN Nutritional Biochemicals, Cleveland, Ohio) per ml to enhance infectivity (10). Virus was extracted from cells and supernatant fluids by homogenization for 1 min at 8,000 rpm, using a Sorvall Omnimixer (Dupont Co., Newtown, Conn.) with 20% Freon 113, separating the Freon phase by low-speed centrifugation, and freezing the supernatant aqueous phase at -90°C until used. SA11 virus titer

determinations were made by immunofluorescent cell counts on cover slips (2, 16). Stock murine rotavirus was prepared by passage into 2- to 3-day-old infant mice, allowing the infection to proceed for 4 to 6 days, and then making a 1:10 homogenate of infected mouse intestines in Earle balanced salt solution. Homogenates were frozen at -90°C until used. The murine rotavirus RNA band patterns were determined and compared with similarly processed RNA of a recent human rotavirus isolate and of the Nebraska strain of bovine rotavirus, using a polyacrylamide gel electrophoresis technique reported previously (1). The ethidium bromide staining procedure of Kalica et al. (12) was used to increase the sensitivity of RNA band detection.

Cells. Embryonic rhesus monkey kidney (MA-104) cells, obtained from Mary Estes, were used in the *in vitro* studies. The original source of these cells was Microbiological Associates. The cell growth medium was Earle minimum essential medium supplemented with 5% heat-inactivated fetal bovine serum (Sterile Systems, Logan, Utah), 0.19% NaHCO_3 , and 50 μg of gentamicin (Schering Corp., Bloomfield, N.J.) per ml. Medium used for the antiviral tests was the same without fetal bovine serum. One μg of trypsin per ml was also present in the medium.

Virus titer reductions. SA11 rotavirus at an input multiplicity of 0.1 infectious units per cell was grown in 24-well microplates (Falcon, Division of BioQuest, Oxnard, Calif.) for 24 h in the presence of drug and drug-free controls. At the end of incubation, cells and supernatants were freeze-thawed, sonicated for 30 s, and refrozen at -90°C until titrated. Resultant fluids were titrated for virus in MA-104 cells by the Reed-Muench end point dilution method (20), using four wells of a 96-well Nunclon microplate (Vanguard International, Neptune, N.J.) per dilution. Presence of virus was determined by cytopathic effect (CPE) induced in the cells. Cells exhibiting no CPE were examined by immunofluorescence.

Biochemical cytotoxicity determinations. To assess the effects of drug on RNA synthesis in uninfected MA-104 cells, 2.5×10^5 cells per well in a 24-well microplate were grown for 18 h, treated with drug for 23 h, and then pulsed for 1 h with 2 μCi of [^3H]uridine or [^3H]adenosine per ml (for 3-DU experiments). These and all other radioisotopes used for studies in this report were purchased from ICN Chemical and Radioisotope Division, Irvine, Calif. Both the trichloroacetic acid-soluble and -insoluble portions were assayed by liquid scintillation, using methods we have described previously (24). A continuous labeling assay was also performed, using ribavirin at 320 $\mu\text{g}/\text{ml}$ and (S)-DHPA at 1,000 $\mu\text{g}/\text{ml}$ in [^3H]uridine-treated cells (10 μCi of [^3H]uridine per ml added concurrently with drug). Every 2 h, four wells of drug-treated cells and eight placebo wells of MA-104 cells in 96-well microplates (24) were solubilized with 10% sodium dodecyl sulfate. After solubilization of all wells, the cells in solution were precipitated with 20% trichloroacetic acid and processed for liquid scintillation analysis.

Dense and light particle synthesis. Particle synthesis experiments were performed by using confluent monolayers of MA-104 cells (10^7 cells per dish) in 100-mm petri dishes (Falcon), an infecting SA11 rotavirus titer of 1 infectious unit per cell, and drug or drug-free medium containing 5 μCi of [^3H]uridine per ml [for 3-

DG and (S)-DHPA] or 5 μCi of [^3H]adenosine per ml (for ribavirin and 3-DU). After 24 h of incubation, cells were scraped off and the cells and supernatants were Freon extracted as described above. Virus was then centrifuged into preformed cesium chloride gradients, and gradients were fractionated by bottom displacement by a recently published procedure (5).

Study of SA11 rotavirus polypeptide synthesis. Virus at 1 infectious unit per cell was propagated in 24-well microplates with or without drug and pulse-labeled for 11 to 13 h after infection with 50 μCi of [^3H]amino acids per ml in amino acid-free medium. Cells were then solubilized with a solution containing 8 M urea, 2% β -mercaptoethanol, and 1% sodium dodecyl sulfate (50 μl per well). Samples were boiled for 1 min, and then 10 μl of each sample was loaded on 9% sodium dodecyl sulfate-polyacrylamide gels prepared by the method of Laemmli (15). After being run, gels were prepared for fluorography (3).

SA11 RNA polymerase assay. Experiments were performed by the method of Cohen et al. (6), using 2 μg of EDTA-treated purified L rotavirions per 0.3 ml of total reaction mixture in 0.1 M Tris-hydrochloride (pH 8.0) buffer. [^3H]uridine triphosphate was used to label the RNA produced. Antiviral drugs were present at a concentration of 500 μM in the mixture. After 1 h of incubation at 37°C , four 25- μl samples of each sample were placed into 50 μl of Tris buffer containing 50 μg of carrier RNA and then precipitated with 1 ml of 10% trichloroacetic acid. Four additional 25- μl samples were placed in 20 μg of RNase (Sigma Chemical Co., St. Louis, Mo.) per ml. After 30 min, the RNase-treated samples were precipitated as described above. Filtration of all precipitates and counting by liquid scintillation were performed as described previously (24). RNA polymerase activity was expressed as RNase-sensitive, acid-insoluble counts per minute.

Mouse studies. Two- to three-day-old Swiss-Webster specific pathogen-free (CrI:CFW^R/SW) mice (Charles River Breeding Laboratories, Wilmington, Mass.) were infected by oral gavage with intestinal homogenates (10 μl per mouse) containing approximately a 60% lethal virus dose. The mice received drug or placebo orally 2 h before infection and then twice daily for 7 days, using 40 mg of ribavirin triacetate per kg per day, 20 mg of (S)-DHPA per kg per day, 0.5 mg of 3-DG per kg per day, or distilled water placebo. Doses selected represented the highest drug concentrations which did not cause death in previously performed toxicity experiments. Approximate 50% lethal doses of the compounds (determined with 6 mice per drug dilution) were 80 mg/kg per day for ribavirin triacetate and (S)-DHPA and 5 mg/kg per day for 3-DG. Litter sizes were adjusted at birth to contain 8 to 10 mice per litter, and every litter had representatives treated with each drug or placebo. Mice under a particular drug treatment were weighed separately each day, and average weights were determined for all mice in the category. Deaths and weight gains were recorded for 21 days. The presence or absence of diarrhea was evaluated on days 3 and 4 of the infection. All drugs and controls were run in parallel.

RESULTS

The effect of antiviral agents on SA11 rotavirus production in MA-104 cells is shown in

TABLE 1. Effect of antiviral substances on total infectious simian rotavirus yields in MA-104 cells^a

Drug concn ($\mu\text{g/ml}$)	Total infectious virus yield ($\log_{10}$ CCID ₅₀ ^b /0.1 ml)			
	Ribavirin	3-DG	3-DU	(S)-DHPA
10	5.5	4.0	5.5	5.7
100	5.0	2.3	5.5	3.7
1,000	3.3	1.7	4.5	2.7

^a The total infectious virus yield for virus control samples 1, 2, and 3 were 5.7, 5.5, and 5.7 $\mu\text{g/ml}$, respectively.

^b CCID₅₀, Fifty percent cell culture infectious dose.

Table 1. These results correlate with CPE inhibition data published previously (24) in that 3-DG had the strongest antiviral activity and 3-DU the least. (S)-DHPA exhibited a greater inhibition of total infectious virus yield than may have been predicted from the previous CPE inhibition experiments.

The data shown in Table 2, which relate cellular RNA synthesis to cytotoxicity, indicate that ribavirin and (S)-DHPA markedly inhibited [³H]uridine uptake in both acid-soluble and -insoluble fractions. Weak inhibitory effects were observed in 3-DG-treated cells, and 3-DU showed a marginal inhibition of uptake of [³H]adenosine at 1,000 $\mu\text{g/ml}$ only. It was not possible to use [³H]uridine as an RNA precursor for 3-DU studies, because the label was completely excluded from the cells treated with 100 $\mu\text{g/ml}$ or greater concentrations of 3-DU. In cells treated with ribavirin or (S)-DHPA and continuously labeled with [³H]uridine (Table 3), inhibition of acid-soluble and -insoluble counts per minute was evident as early as 2 to 4 h after starting the experiment.

The effect of drugs on dense (D) and light (L) SA11 particle synthesis is shown in Fig. 1. The D peak corresponds to precursor particles possessing three to four structural polypeptides, whereas the L peak represents complete rotavirions containing five or eight structural polypeptides (8, 29). Primarily L particles were produced in this cell line. There was a decrease in the sizes of the light particle peaks and no significant buildup of dense particles, suggesting that the drugs did not interfere with D to L conversion, and therefore did not affect viral assembly.

In polyacrylamide gel electrophoresis studies (Fig. 2), the far left lane shows the polypeptide band pattern of uninfected MA-104 cells. Adjacent to this is the lane representing virus-infected cell cultures. At high multiplicities of infection, cellular polypeptide synthesis was greatly inhibited, and only viral polypeptides were observed. The same band pattern is evident in all other lanes except those representing cultures

TABLE 2. Effects of antiviral substances on [³H]uridine or [³H]adenosine uptake and incorporation in MA-104 cells^a

Drug concn ($\mu\text{g/ml}$)	[³ H]adenosine uptake and incorporation (cpm ^b) in cells treated with indicated type of antiviral substances ^c							
	Ribavirin		3-DG		3-DU		(S)-DHPA	
	Acid soluble	Acid insoluble	Acid soluble	Acid insoluble	Acid soluble	Acid insoluble	Acid soluble	Acid insoluble
0	71.3 (100 $\pm$ 3)	19.1 (100 $\pm$ 3)	74.5 (100 $\pm$ 2)	19.8 (100 $\pm$ 5)	203.7 (100 $\pm$ 2)	43.2 (100 $\pm$ 6)	69.1 (100 $\pm$ 3)	17.1 (100 $\pm$ 4)
1	72.6 (102 $\pm$ 1)	20.7 (108 $\pm$ 2)	74.0 (99 $\pm$ 2)	18.3 (92 $\pm$ 8)	211.8 (104 $\pm$ 4)	42.4 (98 $\pm$ 7)	67.8 (98 $\pm$ 4)	18.4 (108 $\pm$ 6)
10	19.1 (27 $\pm$ 1)	3.9 (20 $\pm$ 8)	59.4 (80 $\pm$ 4)	17.9 (90 $\pm$ 7)	197.7 (97 $\pm$ 1)	44.6 (103 $\pm$ 12)	63.9 (92 $\pm$ 1)	15.1 (88 $\pm$ 6)
100	15.2 (21 $\pm$ 2)	2.7 (14 $\pm$ 4)	52.6 (71 $\pm$ 2)	16.4 (83 $\pm$ 4)	190.1 (93 $\pm$ 3)	44.8 (104 $\pm$ 4)	53.9 (78 $\pm$ 1)	12.7 (74 $\pm$ 11)
1,000	24.4 (34 $\pm$ 1)	5.4 (28 $\pm$ 4)	50.3 (68 $\pm$ 1)	16.7 (84 $\pm$ 11)	179.6 (88 $\pm$ 3)	33.1 (77 $\pm$ 4)	16.2 (23 $\pm$ 2)	6.0 (35 $\pm$ 1)

^a [³H]adenosine was used with 3-DU only, owing to drug markedly inhibiting [³H]uridine uptake into cells. An 18-h established monolayer treated with each compound for 23 h, followed by a 1-h pulse.

^b Counts per minute $\times 10^{-3}$; average of eight determinations for drug-free controls and four determinations per dose of drug.

^c Percentage of control $\pm$ standard error of the mean.

TABLE 3. Effects of ribavirin and (S)-DHPA on [³H]uridine uptake and incorporation into MA-104 cells^a under continuous labeling conditions

Hours ^d	[³ H]adenosine uptake and incorporation (cpm ^b) in cells treated with: ^c			
	Ribavirin (320 mg/ml)		(S)-DHPA (1,000 mg/ml)	
	Acid soluble	Acid insoluble	Acid soluble	Acid insoluble
2	71 ± 5 ^e	95 ± 5	56 ± 5	59 ± 7
4	67 ± 5	49 ± 11	60 ± 3	49 ± 5
6	64 ± 7	50 ± 6	67 ± 3	59 ± 8
8	64 ± 4	41 ± 7	66 ± 4	50 ± 5
10	68 ± 3	52 ± 7	64 ± 3	56 ± 5

^a An 18-h established monolayer.

^b Counts per minute, average of four determinations.

^c Percentage of control ± standard error of the mean.

^d Hours after application of drug and radiolabel.

treated with 100 and 1,000 µg of ribavirin or 3-DG per ml. These lanes show an inhibition in viral polypeptides and a reappearance of cellular polypeptides.

The results of studies of the inhibition of SA11 rotavirus RNA polymerase by the various drugs

are shown in Table 4. None of the agents evaluated influenced viral polymerase activity by more than ±20% (*P* > 0.05) of the control, indicating that they caused no significant inhibition of the enzyme.

Figure 3 shows a comparison of virus RNA

FIG. 1. Effect of antiviral substances on D and L simian rotavirus particle synthesis in MA-104 cells. Drug concentrations used: ribavirin, 100 µg/ml; 3-DG, 10 µg/ml; 3-DU, 100 µg/ml; and (S)-DHPA, 100 µg/ml. D particle peak around fraction 8; L particle peak standardized at fraction 15.

FIG. 2. Effect of antiviral substances on simian rotavirus polypeptide synthesis in MA-104 cells. ^aCC, cell control; ^bVC, virus control; and ^cValues for 1, 10, and 100 are given as micrograms per milliliter times 10.

band patterns for murine, human, and bovine rotaviruses. Although differing in minor aspects, as would be expected for different species isolates, the band patterns were sufficiently similar to indicate that the murine virus is a rotavirus.

In the *in vivo* studies, none of the agents caused a reduction in the number of mice with discernible diarrhea or caused an increase in the total number of survivors. However, ribavirin triacetate and (S)-DHPA increased mean survival time significantly. 3-DG- and (S)-DHPA-treated infected mice gained weight at a rate approaching that of the normal uninfected control (Fig. 4), suggesting that the drugs may have lessened the severity of the disease in survivors. Because of the high degree of variation in weight among mice of the same category, however, no

statistical significance between groups could be established. Ribavirin triacetate may have been marginally toxic at the dose given, based upon one death and low weight gain observed in the uninfected, untreated animals.

DISCUSSION

The dose-response relationship between concentration of drug used and development of total infectious rotavirus particles correlated with degrees of CPE inhibition and immunofluorescent cell count reduction observed in our previous studies (24). Although 3-DG caused the greatest degree of viral inhibition, (S)-DHPA was considered to be more effective because of its lower toxicity *in vivo*.

The effect of drug on [³H]uridine uptake indicated that ribavirin, 3-DG, and (S)-DHPA inhibited both acid-soluble and -insoluble fractions, and the degree of inhibition of the two fractions was nearly the same. Canonico et al. (4) reported a similar effect of ribavirin on [³H]uridine uptake in baby hamster kidney (BHK-21) cells; they concluded that ribavirin altered the intracellular uridine pool size but did not inhibit cellular RNA synthesis *per se*. With less [³H]uridine being taken into drug-treated cells, decreased labeling of RNA would be expected even though RNA synthesis could be proceeding at a normal rate. Since under continuous labeling conditions the inhibition of acid-soluble [³H]uridine counts per minute was observed early in the course of treatment, the decrease in uptake of radiolabel cannot be attributed to merely having fewer viable cells in drug-treated wells compared with controls. Other biochemical studies on the effects of these compounds on cellular and DNA synthesis were presented previously (24). Another antiviral substance, arilone, also inhibits [³H]uridine uptake into acid-soluble and -insoluble pools (18).

The remaining *in vitro* studies were designed to elucidate possible mechanisms of drug action against SA11 rotavirus. In studying the effects of drug on virus assembly, it appears that none of the compounds affect the conversion of D (precursor) to L (complete) rotavirions. If an agent were to inhibit this conversion, one would have expected to see a large D and a reduced L particle peak, i.e., a shift in the amount of D relative to L particles. The sizes of the D and L peaks for drug-treated cultures may have been reduced because of the inhibition of ³H-precursor uptake into the cell, but this would not affect the relative amounts of D and L particles.

Much can be inferred from the acrylamide gel electrophoresis results that show inhibition of SA11 polypeptides by ribavirin and 3-DG and the lack of inhibition by 3-DU and (S)-DHPA. Because viral polypeptides are a product of

TABLE 4. Effect of antiviral substances on simian rotavirus RNA polymerase activity

Compound ^a	cpm ^b × 10 ⁻³ ± standard error	Percentage of control
Control	14.5 ± 9	100
Ribavirin	13.3 ± 10	91
Ribavirin 5'-monophosphate	15.4 ± 11	106
Ribavirin 5'-triphosphate	13.2 ± 8	91
3-DG	12.6 ± 7	87
3-DU	13.7 ± 6	93
(S)-DHPA	17.2 ± 7	118

^a 500 μM concentration for each drug.

^b RNase-digestible acid-insoluble counts per minute.

FIG. 3. Comparison of RNA band patterns of murine (MRV), human (HRV), and bovine (BRV) rotaviruses.

FIG. 4. Effect of antiviral substances on average weights for uninfected (open bars) and murine rotavirus-infected (hatched bars) mice 21 days after initiation of treatment. Mice weighing 2 to 3 g at the start of the experiment received drugs twice a day for 7 days.

translated viral mRNA, it is probable that ribavirin and 3-DG affect either the quantity or quality of viral mRNA. The possibility that ribavirin or its mono- and triphosphate derivatives affect the quantity of viral mRNA by directly inhibiting the SA11 RNA polymerase can be ruled out by the data presented in Table 3. The phosphorylated derivatives of 3-DG were not available for evaluation, however, so a direct inhibition of rotavirus polymerase by derivatives of 3-DG remains a possibility (27).

Two other mechanisms of viral mRNA inhibition by ribavirin and 3-DG have been proposed in the literature. The first mechanism is the inhibition of cellular inosine 5'-monophosphate dehydrogenase by both agents (27, 28), which, by lowering nucleotide levels intracellularly, could inhibit viral RNA synthesis in a nonspecific manner. Canonico and colleagues (4) recently have shown nucleotide levels in uninfected and Venezuelan equine encephalitis virus-infected cells to be the same, which suggests that inosine 5'-monophosphate dehydrogenase inhibition is not the primary mechanism of Venezuelan equine encephalitis virus inhibition. If a decrease in nucleotide levels in cells causes an inhibition of viral RNA synthesis, one would expect this decrease to inhibit cellular RNA synthesis as well, since the mechanism is nonspecific. But, for 3-DG especially (Table 2), the effect of drug on [³H]uridine uptake is marginal at concentrations where SA11 virus production (Table 1) and viral polypeptide synthesis (Fig. 2) are markedly inhibited. These data suggest that the inhibition of inosine 5'-monophosphate dehydrogenase is unrelated to antirotavirus activity.

Another mechanism of virus inhibition by ribavirin is the inhibition of capping of viral messenger RNA reported for vaccinia (9) and Venezuelan equine encephalitis (4) viruses. The lack of capping would render viral mRNA nonfunctional for translation to polypeptides but would not necessarily reduce the total amount of viral mRNA produced. Whether mRNA capping inhibition is the principal mechanism of SA11 rotavirus inhibition by ribavirin or 3-DG remains to be determined.

The mechanism of action of 3-DU and (S)-DHPA against other viruses has not been elucidated (23), but (S)-DHPA is believed to inhibit the enzymatic activity of Rous sarcoma virus protein kinase (14) and vaccinia virus mRNA synthesis (19). It is concluded from studies presented here that these agents do not inhibit simian rotavirus polypeptide synthesis and may not affect viral mRNA production, since polypeptides are translated from mRNA. No inhibition of viral RNA polymerase activity was evident when these compounds were present in the

reaction mixture. Phosphorylated derivatives of 3-DU, which may be the biologically active forms (23), were unavailable for testing. The biologically active form of (S)-DHPA is most likely the form used for the polymerase assay, based upon its stability in vivo (14). Virion assembly was not affected by these agents (Fig. 1). The remaining possibility is that 3-DU and (S)-DHPA inhibit double-stranded genomic RNA synthesis. The possibility that ribavirin and 3-DG also inhibit double-stranded RNA synthesis cannot be ruled out either.

The antiviral activities observed against murine rotavirus infection were moderate at best. There are several possible explanations for the weak activity of the compounds against this disease. First, the mice only tolerated low concentrations of the drug for the length of treatment employed. We have used ribavirin triacetate and (S)-DHPA at much higher doses (500 mg/kg per day) in adult animals, but these infant mice were more sensitive to the compounds under these treatment conditions. Another possible explanation for the low degree of drug efficacy may be a drug distribution problem wherein a sufficient amount of agent was not present at the target organ to inhibit viral replication in the intestinal epithelial cells. A third possibility was suggested by Schoub and Prozesky (22), who concluded that the antiviral activity of ribavirin may be reversed by the production of guanosine by mononucleotidases in the guts of mice. Since guanosine and other natural nucleotides are produced in the intestinal tract (11), a reversal of ribavirin triacetate, 3-DG, and (S)-DHPA activity by the substances may have occurred. Were these agents to remain in the intestinal tract for any length of time, they could also be metabolized (deaminated) to inactive forms by microbial enzymes (21).

Of the four antiviral agents evaluated in this report, (S)-DHPA appears to have the greatest potential for use as an antirotavirus inhibitor in humans. The conclusion is based upon the marked effect of drug in vitro on virus production, the moderate in vivo effect of drug seen in these studies, and the low toxicity in larger animals as reported by others (7, 14).

There is a clinically prolonged course of the rotaviral disease in many infants, caused by continued replication of virus in superficial epithelial cells of the small intestine. Theoretically, an antiviral agent, if administered relatively early in the infection, may be able to inhibit these cumulative effects of intestinal epithelial damage. Rotavirus vaccines are an alternative to using antiviral agents to control the disease, but these vaccines are still in the experimental stage of development (25, 30). Vaccines are prophylactic rather than therapeutic, whereas antiviral

substances may be both. The results presented in this and in previous reports (22, 24) may serve as a basis for evaluating other, more specific rotaviral inhibitors.

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LITERATURE CITED

- Barnett, B. B., L. N. Egbert, and R. S. Spendlove. 1978. Characteristics of neonatal calf diarrhea virus ribonucleic acid. *Can. J. Comp. Med.* 42:46-53.
- Barnett, B. B., R. S. Spendlove, M. W. Peterson, L. Y. Hsu, V. A. LaSalle, and L. N. Egbert. 1975. Immunofluorescent cell assay of neonatal calf diarrhea virus. *Can. J. Comp. Med.* 39:462-465.
- Bonner, W. M., R. A. Lasky. 1974. A film detection method for tritium labelled proteins and nucleic acids in polyacrylamide gels. *Eur. J. Biochem.* 46:83-88.
- Canonica, P. G., J. S. Little, P. B. Jahrling, and E. L. Stephen. 1980. Molecular aspects of the antiviral activity of ribavirin on Venezuelan equine encephalomyelitis virus, p. 1370-1372. *In* J. D. Nelson and C. Grassi (ed.), *Current chemotherapy and infectious disease*. vol. II. American Society of Microbiology, Washington, D.C.
- Clark, S. M., R. S. Spendlove, and B. B. Barnett. 1980. Role of two particle types of bovine rotavirus morphogenesis. *J. Virol.* 34:272-276.
- Cohen, J., J. LaPorte, A. Charpillienne, and R. Scherrer. 1979. Activation of rotavirus RNA polymerase by calcium chelation. *Arch. Virol.* 60:177-186.
- DeClercq, E., J. Descamps, and P. DeSomer. 1978 (S)-9-(2,3-dihydroxypropyl)adenine: an aliphatic nucleoside analog with broad spectrum antiviral activity. *Science* 200:563-564.
- Espejo, R. T., S. Lopez, and C. Arias. 1981. Structural polypeptides of simian rotavirus SA11 and the effect of trypsin. *J. Virol.* 37:156-160.
- Goswami, B. B., E. Borek, and O. K. Sharma. 1979. The broad spectrum antiviral agent ribavirin inhibits capping of mRNA. *Biochem. Biophys. Res. Commun.* 89:830-836.
- Graham, D. Y., and M. K. Estes. 1980. Proteolytic enhancement of rotavirus infectivity: biological mechanisms. *Virology* 101:432-439.
- Harper, H. A. 1969. *Review of physiological chemistry*, p. 373. Lange Medical Publications, Los Altos, Calif.
- Kalica, A. R., C. F. Garon, R. G. Wyatt, C. A. Mebus, D. M. VanKirk, R. M. Chanock, and A. Z. Kapikian. 1976. Differentiation of human and calf reovirus-like agents associated with diarrhea using polyacrylamide gel electrophoresis of RNA. *Virology* 74:86-92.
- Kapikian, A. Z., R. M. Yolken, R. G. Wyatt, A. R. Kalika, R. M. Chanock, and H. W. Kim. 1978. Viral diarrhea: etiology and control. *Am. J. Clin. Nutr.* 31:2219-2236.
- Kara, J., P. Vacha, and A. Holy. 1979. 9-(S)-(2,3-dihydroxypropyl)adenine inhibits the transformation of chick embryo fibroblasts infected with Rous sarcoma virus. *FEBS Lett.* 107:187-192.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 227:680-685.
- McClain, M. E., R. S. Spendlove, and E. H. Lennette. 1967. Infectivity assay of reoviruses: comparison of immunofluorescent count and plaque methods. *J. Immunol.* 98:1301-1308.
- McNulty, M. S. 1978. Rotaviruses. *J. Gen. Virol.* 40:1-18.
- McSharry, J. J., L. A. Caliguiri, and H. J. Eggers. 1979. Inhibition of uncoating of poliovirus by arildone, a new antiviral drug. *Virology* 97:307-315.
- Rada, B., M. Dragun, I. Votruba, and A. Holy. 1980. Characteristics of the antiviral effect of 9-(S)-(2,3-dihydroxypropyl)adenine. *Acta Virol.* 24:433-438.
- Reed, L. J., and M. Muench. 1938. A simple method of estimating fifty percent end points. *Am. J. Hyg.* 27:493-498.
- Schelline, R. R. 1973. Metabolism of foreign compounds by gastrointestinal microorganisms. *Pharmacol. Rev.* 25:451-523.
- Schoub, B. D., and O. W. Prozesky. 1977. Antiviral activity of ribavirin in rotavirus gastroenteritis of mice. *Antimicrob. Agents Chemother.* 12:543-544.
- Sidwell, R. W., and J. T. Witkowski. 1979. Antiviral agents, p. 543-593. *In* M. E. Wolff (ed.), *Burger's Medicinal Chemistry*, 4th edition, part II. John Wiley & Sons, Inc., New York.
- Smee, D. F., R. W. Sidwell, B. B. Barnett, and R. S. Spendlove. 1981. Inhibition of rotaviruses by selected antiviral substances, p. 123-138. *In* S. D. Acres, A. J. Forman, and H. Fast (ed.), *Proceedings of the Third International Symposium of Neonatal Diarrhea*. Veterinary Infectious Disease Organization, Saskatoon, Saskatchewan, Canada.
- Snodgrass, D. R., K. J. Fahey, P. W. Wells, I. Campbell, and A. Whitelaw. 1980. Passive immunity in calf rotavirus infections: maternal vaccination increases and prolongs immunoglobulin G1 and antibody secretion in milk. *Infect. Immun.* 28:344-349.
- Spendlove, R. S. 1978. Reoviridae: rotaviruses, p. 251-265. *In* D. Seligson, G. D. Hsiung, and R. H. Green (ed.), *Handbook series in clinical laboratory science, section H: virology and rickettsiology*, vol. I, part I. CRC Press, West Palm Beach, Florida.
- Streeter, D. G., and H. H. P. Koyama. 1976. Inhibition of purine nucleotide biosynthesis by 3-deazaguanine, its nucleoside and 5'-nucleotide. *Biochem. Pharmacol.* 25:2413-2415.
- 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 -D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Virazole), a new broad spectrum antiviral agent. *Proc. Natl. Acad. Sci. U.S.A.* 70:1174-1178.
- Thouless, M. E. 1979. Rotavirus polypeptides. *J. Gen. Virol.* 44:187-197.
- Wells, P. W., D. R. Snodgrass, J. A. Herring, and A. M. Dawson. 1978. Antibody titres to lamb rotavirus in colostrum and milk and vaccinated ewes. *Vet. Rec.* 103:46-48.