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Evaluation of the toxicity and antiviral activity of carbocyclic 3-deazaadenosine against respiratory syncytial and parainfluenza type 3 viruses in tissue culture and in cotton rats

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Summary

The toxicity and antiviral efficacy of carbocyclic 3-deazaadenosine (Cc3Ado) against respiratory syncytial (RSV) and parainfluenza type 3 (PIV3) virus infections were tested in tissue culture and in cotton rats. The mean median efficacious dose (ED_{50}) of Cc3Ado in HEp2 cells against RSV and PIV3 was 9 and 14 μg/ml, respectively. These values were 85- and 55-fold less than the median inhibitory (toxic) dose (ID_{50}) of Cc3Ado in this cell line (750 μg/ml), and similar to values obtained for ribavirin. Cc3Ado exhibited no significant antiviral activity against influenza A, influenza B, adeno type 5 or adeno type 7 viruses (all ED_{50} were >1000 μg/ml). In cotton rats, animals given $\geq$ 1 mg/kg/day Cc3Ado intraperitoneally on days 1, 2 and 3 after experimental challenge with virus, consistently had significant reductions in pulmonary RSV and PIV3 titers compared to pulmonary virus titers in comparably treated control animals. The minimum efficacious dose of ribavirin given under the same conditions was 30 mg/kg/day. Cc3Ado was also efficacious in cotton rats when given orally by gavage, or when different administration schedules were used. The median efficacious dose of Cc3Ado when given orally was 10 mg/kg/day. No significant toxic effects were noted in cotton rats, even in animals given 20 mg/kg daily for eight consecutive days.

Respiratory syncytial virus; Parainfluenza virus type 3; Carbocyclic 3-deazaadenosine; Cotton rat

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Introduction

Respiratory syncytial (RSV) and parainfluenza type 3 (PIV3) viruses are leading causes of serious lower respiratory tract infections in children under two years of age (Channock et al., 1959; Glezen et al., 1982). No vaccines are currently approved for use against either virus. Moreover, no antivirals are licensed for use against PIV3, and the only compound approved for use against RSV, ribavirin, may only be used when delivered by continuous small particle aerosol. Thus treatment with this drug is both expensive and practical only for use against severe disease. Identification and development of other compounds with antiviral activity against these viruses that could be administered parenterally or orally would be highly desirable.

Carbocyclic 3-deazaadenosine (Cc3Ado) and its parent compound, 3-deazaadenosine, have been reported to inhibit the replication of paramyxoviruses (i.e., measles and PIV3) in vitro (Chiang, et al., 1977; De Clercq and Montgomery, 1983). To our knowledge these compounds have not been tested in vivo against any member of the paramyxoviridae. In this report, we describe studies testing and comparing the toxicity and antiviral activity of Cc3Ado in tissue culture and in cotton rats against RSV and PIV3. Cc3Ado exhibited little toxicity in cultured cells or in cotton rats, but did significantly inhibit replication of RSV and PIV3 in both HEp2 cells and in cotton rats.

Materials and Methods

Animals

All cotton rats (*Sigmoden fulviventer*) used in these studies were bred from several pair obtained from Dr Gregory Prince and the Small Animal Section, Veterinary Research Branch, Division of Research Services, National Institutes of Health. Test animals were between 50 and 100 g at the start of each experiment and of either sex. All animals were maintained in cages with barrier filters and fed water and food ad libitum.

Tissue culture

HEp2 (ATCC CCL23), L929 (ATCC CCL10), WI38 (ATCC CCL 75), Madin Darby canine kidney (MDCK; ATCC NBL-2) and A549 (ATCC CCL 185) tissue culture cells were obtained from the American Type Culture Collection (ATCC). Whenever any flasks containing these cell lines became confluent, they were serially passaged using Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS), penicillin (100 units/ml), streptomycin (100 μg/ml), sodium bicarbonate (0.2%), and L-glutamine (2 mM).

Viruses

Seed parainfluenza virus type 3 (PIV3; ATCC VR93) was obtained from the ATCC. The RSV used in these studies was obtained originally from a patient hospitalized with a severe respiratory infection. Its identity as a type A RSV was made by Dr Larry Anderson of the Communicable Disease Center, Atlanta, GA, using a battery of virus-specific monoclonal antibodies.

Both the RSV and PIV3 used formed distinctive and characteristic syncytia in HEp2 tissue cells. Stocks of both viruses were prepared by infecting monolayers of HEp2 cells. When these monolayers exhibited approximately 90% syncytia formation, the medium from the monolayers was collected, pooled and clarified by centrifugation (450 × *g*). The clarified supernatant fluid obtained was passed through a 0.45 μM filter (Acrodisc, cat. no. 4184, Gelman, Ann Arbor, MI), portioned and stored at −70°C.

The influenza A/Taiwan/86 (H1N1), A/Leningrad/86 (H3N2) and B/USSR/83 viruses used in these studies were obtained from the Influenza Research Center, Baylor College of Medicine, Houston, TX. Working pools of these viruses were prepared by inoculating flasks of MDCK cells with the appropriate virus and waiting until ≥ 90% of the infected flasks exhibited cytopathic effects (CPE). The medium used to support influenza virus growth was 0% FCS-MEM containing 2 μg/ml (final concentration) trypsin (Worthington Biochemicals, Freehold, N.J.). When ≥ 90% CPE was observed, the medium from each flask was removed, clarified, portioned, labelled and frozen at −70°C until used.

Adenovirus type 5 (AV5) and type 7 (AV7) were obtained from Dr Julius Kasel, Department of Microbiology and Immunology, Baylor College of Medicine, Houston, TX. Working pools of these two viruses were prepared by inoculating flasks of A549 cells with AV5 or AV7 and waiting until ≥ 90% of the infected flasks exhibited CPE. The medium from each infected flask was then removed, clarified, portioned, labelled and frozen at −70°C until used.

Compounds

Cc3Ado was obtained from Drs John Montgomery and John A. Sechrist III, of Southern Research Institute, Birmingham, AL and was a gift from ViraChem, Inc., Birmingham. The compound is a racemic mixture. Its structure, major characteristics and probable mechanism(s) of antiviral action have been described in detail (Montgomery et al., 1982; De Clercq and Montgomery, 1983). Ribavirin was obtained from Viratek, Inc., Covina, CA. Initial concentrations of both compounds were made in sterile distilled water. Additional dilutions were made in 2% FCS-MEM.

Virus quantification

Assays to detect and quantify virus in different preparations were generally performed in 96-well tissue culture plates (Linbro). In these assays, serial half

$\log_{10}$ dilutions of each virus sample were made in 2% FCS-MEM. Approximately 3×10^3 HEp2 cells were then added to each well. The plates were placed in a 35°C, 5% CO_2 incubator for seven days. Wells were observed daily for formation of syncytia or other CPE. Mean virus titers ($\log_{10}$/g of lung or /ml of fluid) were determined by calculating the means of the last dilutions in replicate rows that contained virus.

Cytotoxicity in vitro

Cc3Ado and ribavirin were tested for cytotoxicity in quadruplicate in 96-well flat bottom tissue culture plates (Falcon 3072). In these assays, each test compound was added to the initial wells so that the final concentration of drug in these wells was 1 mg/ml in 2% FCS-MEM. Vehicle (H_2O) controls were included in each assay. The Cc3Ado, ribavirin and vehicle control were then diluted in 2% FCS-MEM using serial 2-fold dilutions. Depending on which cell line was being tested, approximately 3×10^3 HEp2, L929, WI38, A549 or MDCK tissue cells were added to each well, including wells that contained just medium (cell control). At the concentration of cells used in these assays, the monolayers that formed following settlement and attachment of cells were 20 to 30% confluent. Wells containing the cell controls were observed daily. When cells in these wells reached confluency, all wells were observed for cytopathic effects (CPE) or inhibition of monolayer formation. Toxicity was considered to have occurred if >20% of the cells in a monolayer exhibited CPE or if monolayer remained <50% confluent. The median inhibitory (toxic) dose (ID_{50}) was calculated (in μg/ml) for each cell type by determining the mean concentration of test compound in the last wells of replicate rows exhibiting >20% CPE or <50% confluency.

Antiviral activity in vitro

Assays to assess the antiviral activity of Cc3Ado and ribavirin in tissue culture were performed in 96-well flat bottom tissue culture plates (Falcon 3072). In these assays, Cc3Ado or ribavirin were added to initial wells at a final concentration of 1 mg/ml and serially diluted (2-fold) in 2% FCS-MEM (0.05 ml/well). A 0.05 ml volume of the appropriate virus containing about 100 median tissue culture infectious doses ($TCID_{50}$) was then added to all wells but those set aside as antiviral and tissue control wells. Approximately 3×10^3 HEp2 cells (0.1 ml) was then added to each well. Control wells containing antiviral and no virus (antiviral control), containing virus but no antiviral (virus control), or containing medium without virus or antiviral (tissue control), were included in each test. A back titration of each challenge virus was also run with each assay.

All assay plates were incubated at 35°C for 5 to 7 days in a 5% CO_2 incubator. When virus control wells exhibited 70 to 100% syncytia or other CPE, all wells were observed. The medium efficacious dose (ED_{50}) was calculated after determining the final concentration of antiviral in the last wells of each set of replicate rows exhibiting <50% CPE compared to the CPE in virus control wells.

Collection of lungs

Lungs were removed, weighed and rinsed in sterile saline. Except for those requiring histologic processing, each was transpleurally lavaged using 3 ml of 2% FCS-MEM as described previously (Wilson et al., 1980). Lungs required for histologic examination were similarly removed, but these were placed in buffered formalin and processed as described next.

Histologic methods and evaluation

Lungs were removed for histologic examination and placed in buffered formalin for a minimum of 24 h. They were then embedded in low-melting point paraffin, sectioned at 5 μM thickness, and stained with hematoxylin and eosin. The stained sections were coded and observed in a blinded fashion for evidence of histopathology.

Antiviral activity in vivo

On day 0, all animals were weighed, anesthetized lightly with ether and inoculated intranasally (i.n.) with approximately 100 median cotton rat infectious doses ($CRID_{50}$) of RSV or PIV3 in 0.1 ml. On days +1, +2 and/or +3, all animals were administered 0.1 ml of graded doses of Cc3Ado or ribavirin intraperitoneally (i.p.) or orally (by gavage). Control animals were given sterile water (placebo) similarly. All animals were killed on day +4, the day of maximum RSV or PIV3 pulmonary infection in untreated cotton rats. The lungs of each animal were then removed, weighed and assayed for virus levels as described above.

Toxicity studies in cotton rats

Weight changes, morbidity, mortality and diarrhea in test animals were looked for in all experiments. However, in experiments specifically performed to determine if Cc3Ado had toxic effects in animals, cotton rats were weighed and bled from the retroorbital sinus plexus at the start of each experiment. For the next eight days, each animal was given single i.p. inoculations or oral administrations of placebo (water) or Cc3Ado (20 mg/kg/dose). On day eight, one hour after the last administration of drug, each animal was reweighed, bled and killed. The lungs, liver and kidneys from each animal were removed and placed in formalin for histologic studies. The sera obtained from these animals were sent to Animal Reference Laboratories, Inc., Houston, Texas for determination of blood urea nitrogen (BUN), aspartate transaminase (AST) and alanine transaminase (ALT) levels.

Statistics

Means, standard errors of the means, standard deviations and Student's *t*-tests were calculated using True Epistat, a statistical program designed by T.L. Gustafson

of Epistat Services, Richardson, Texas, for IBM compatible computers. In all comparisons two-tailed analyses were used.

Results

Cytotoxicity in vitro

A comparison of the cytotoxic activity (expressed as ID_{50} values) of Cc3Ado and ribavirin in different tissue culture cell lines is shown in Table 1. Even at the highest concentration of drug tested (1000 μg/ml), Cc3Ado did not cause significant CPE or notably inhibit the growth of A549 human carcinoma cells. Cc3Ado did exhibit some cytotoxicity in each of the other cell lines, but only at relatively high concentrations (ID_{50} = 750 μg/ml). The ID_{50} of ribavirin against all cell types tested was 1000 μg/ml.

Antiviral activity in vitro

Table 1 also compares the antiviral activity (expressed as ED_{50} values) of Cc3Ado and ribavirin. Neither compound was active against adenovirus type 5 (AV5) or 7 (AV7), and only ribavirin had significant antiviral activity against any of the influenza viruses tested (i.e., A/Taiwan, A/Leningrad and B/USSR). Because the ED_{50} values for Cc3Ado against all of these viruses were $\geq$ the ID_{50} values of Cc3Ado in MDCK or A549 cells, the mean selective index (S.I.) or ratio of the ID_{50}/ED_{50} for Cc3Ado against each of these viruses was ≤ 1.

Both Cc3Ado and ribavirin exhibited significant antiviral activity against RSV and PIV3. The mean ED_{50} and S.I. obtained for Cc3Ado in HEp2 cells against RSV was 9 μg/ml and 85, respectively; the ED_{50} and S.I. values obtained for ribavirin against RSV were 11 μg/ml and 93, respectively. Against PIV3, Cc3Ado had a mean ED_{50} value and S.I. of 14 μg/ml and 55, respectively; values of 21 μg/ml and 48 were obtained for ribavirin against this virus.

TABLE 1
Comparison of the in vitro toxicity (ID_{50}) and antiviral activity (ED_{50}) of carbocyclic 3-deaza-adenosine (Cc3Ado) and ribavirin[a]

Cell line	Virus	ID_{50}[b] (μg/ml)		ED_{50} (μg/ml		S.I. (ID_{50}/ED_{50})	
		CcAdo	Rib.	CcAdo	Rib.	CcAdo	Rib.
HEp2	RSV	750	1000	9(9)	11(10)	85	93
HEp2	PIV3	750	1000	14(10)	21(14)	55	48
MDCK	A/Len.	1000	1000	>1000	11(9)	<1	115
MDCK	A/Brazil	1000	1000	>1000	14(10)	<1	72
MDCK	B/USSR	1000	1000	>1000	39(40)	<1	26
A549	AV5	>1000	1000	>1000	>1000	1	<1
A549	AV7	>1000	1000	>1000	>1000	1	<1

[a]Mean values from $\geq$ 3 experiments shown; standard deviations >0 are shown in parentheses.
[b]Abbreviations: ID_{50}, median inhibitory (toxic) dose; ED_{50}, median efficacious dose; RSV, respiratory syncytial virus; PIV3, parainfluenza type 3; A/Len., influenza A/Leningrad (H3N2); A/Brazil, H1N1 influenza; AV5, adenovirus 5; AV7, adenovirus 7.

TABLE 2
Antiviral activity of carbocyclic 3-deazaadenosine (Cc3Ado) in cotton rats against respiratory syncytial virus (RSV)[a]

Expt. no.	Dose Cc3Ado (mg/kg/day)	Dose ribavirin (mg/kg/day)	Pulmonary virus titer[b] in animals given	
			Cc3Ado	Ribavirin
1	Placebo	Placebo	4.1 (0.3)	4.5 (0.2)
	1	10	3.0 (0.3)[c]	4.5 (0.2)
	3	30	3.1 (0.3)	4.0 (0.3)
	10	90	3.0 (0.2)	2.8 (0.3)
2	Placebo	Placebo	4.5 (0.3)	4.5 (0.5)
	0.3	20	3.7 (0.5)	4.8 (0.3)
	1	40	3.5 (0.3)	2.9 (0.4)
	3	80	3.6 (0.5)	2.9 (0.4)
3	Placebo	Placebo	3.5 (0.4)	4.5 (0.5)
	0.1	20	4.1 (0.6)	4.3 (0.5)
	0.3	Not done	3.7 (0.2)	Not done
	1	Not done	2.2 (0.3)	Not done

[a]Cc3Ado or ribavirin was given intraperitoneally on days +1, +2 and +3 after virus challenge; animals were harvested and lungs assayed for RSV on day +4.
[b]Mean pulmonary virus titer (± standard deviation) $\log_{10}$/g lung; number of animals/group = 4.
[c]Underlined values denote mean values with $P<0.03$ when compared to mean values in placebo group using Student's *t*-test.

Activity in vivo

In all three experiments depicted in Table 2, pulmonary RSV titers were significantly less in animals given ≥ 1 mg/kg/day Cc3Ado than in animals given placebo ($P \leq 0.03$ using Student's *t*-test). Reductions in pulmonary RSV titers in groups given 1 mg/kg/day ranged from 1.0 to 1.3 $\log_{10}$. Similar (0.9 to 1.1 $\log_{10}$) significant reductions in pulmonary virus titers were also observed in groups of animals receiving 3 or 10 mg/kg of Cc3Ado daily. In experiment two, animals given 0.3 mg/kg Cc3Ado daily had significantly less pulmonary virus titers than placebo control animals ($P<0.05$; $\log_{10}$ reduction = 0.8). However, in experiment three, no reduction in pulmonary RSV titers was observed in animals given this dose of Cc3Ado daily (3.7 $\log_{10}$ versus 3.5 $\log_{10}$; $P>0.05$). Animals given 0.1 mg/kg of antiviral daily in experiment 3, also did not have significantly reduced pulmonary virus titers. Thus, 1.0 mg/kg/day was the minimum dose of Cc3Ado which consistently inhibited RSV replication in cotton rats in these experiments.

A series of similar experiments were performed to determine the minimum dose of ribavirin which could consistently inhibit RSV replication in cotton rats. As indicated in Table 2, daily i.p. injections of ribavirin of 30 or 40 mg/kg significantly reduced the pulmonary RSV titers of cotton rats compared to pulmonary titers of RSV in animals given placebo (experiments 1 and 2). In contrast, pulmonary virus titers were not significantly reduced in animals given 20 mg/kg/day ribavirin i.p. (experiments 2 and 3). Thus, the minimum efficacious dose of ribavirin in these experiments was 30 mg/kg/day.

Cc3Ado was equally as effective in reducing PIV3 infection in cotton rats. As indicated in Table 3, experiment 1, animals given 1 or 3 mg/kg/day of Cc3Ado

TABLE 3
Antiviral activity of carbocyclic 3-deazaadenosine (Cc3Ado) in cotton rats against parainfluenza virus type 3 (PIV3)[a]

Expt. no.	Dose (mg/kg/day)	Pulmonary virus titer[b] (log_{10}/g lung)
1	Placebo	5.1 (0.4)
	0.3	5.2 (0.1)
	1	3.8 (0.7)[c]
	3	3.7 (0.6)
2	Placebo	3.9 (0.3)
	0.01	3.6 (0.0)
	0.1	3.3 (0.3)
	1.0	3.1 (0.3)

[a]Cc3Ado was given intraperitoneally on days +1, +2 and +3 after virus challenge; animals were harvested and lungs assayed for PIV3 on day +4.
[b]Mean pulmonary virus titer (± standard deviation); number of animals/group = 4.
[c]Underlined values denote mean values with $P<0.05$ when compared to mean values in placebo group using Student's *t*-test.

i.p., had significantly lower pulmonary virus titers than placebo controls (1.3 and 1.4 log_{10} reductions in pulmonary virus titers compared to controls; $P \leq 0.02$ in both instances). However, animals given 0.3 mg/kg Cc3Ado daily did not have a significant reduction in pulmonary PIV3 titers (5.1 log_{10} vs 5.2 log_{10}). In experiment 2, cotton rats given 1 mg/kg/day Cc3Ado again had significantly lower pulmonary PIV3 titers than placebo control animals (0.8 log_{10} reduction in pulmonary virus titer, $P<0.05$); however, no significant reductions in PIV3 pulmonary virus titers were seen in animals administered 0.01 or 0.1 mg/kg/day Cc3Ado daily. Thus, the minimum efficacious dose against PIV3 in cotton rats was 1 mg/kg/day, the same as that obtained against RSV.

Effects of route of administration

A comparison of lung virus titers in RSV-infected cotton rats administered 1 mg/kg/day of Cc3Ado by different routes of administration (i.e., oral, i.p. and i.m.) on days +1 through +3 is shown in experiment 1, Table 4. At 1 mg/kg/day, only drug given i.p. was efficacious. However, as shown in experiment 2, Table 4, significant reductions in pulmonary RSV titers were seen in cotton rats given 10 mg/kg/day Cc3Ado by gavage compared to pulmonary virus titers in placebo control animals treated similarly (1.6 log_{10} reduction in pulmonary virus, $P<0.03$).

Toxicity of Cc3Ado in cotton rats

No overt signs of toxicity (i.e., morbidity, diarrhea, mortality) were observed in any cotton rat involved in these tests. Neither was any evidence of toxicity observed in cotton rats used in experiments designed specifically to look at the toxicity of Cc3Ado. There were no deaths or signs of morbidity in cotton rats administered 20 mg/kg Cc3Ado i.p. or orally for eight consecutive days, nor

TABLE 4
The activity of Cc3Ado in cotton rats against respiratory syncytial virus (RSV) when given by different routes of inoculation[a]

Expt. no.	Dose (mg/kg)	Route of inoculation	Pulmonary RSV titer[b] (log_{10}/g lung)
1	Placebo	Oral (gavage)	4.0 (0.4)
	1	Intraperitoneal	2.6 (0.1)[c]
	1	Intramuscular	3.7 (0.3)
	1	Oral (gavage)	3.7 (0.6)
2	Placebo	Oral (gavage)	4.2 (0.5)
	1	Oral (gavage)	4.0 (0.4)
	3	Oral (gavage)	4.3 (0.3)
	10	Oral (gavage)	2.6 (0.7)

[a]Cc3Ado was given once daily on days +1, +2 and +3 after virus challenge; animals were harvested and lungs were assessed for virus on day +4.
[b]Mean pulmonary virus titer (± standard deviation); number of animals/group = 4.
[c]Underlined values denote mean values with $P \leq 0.03$ when compared to the mean value in the placebo group using Student's *t*-test.

were significant differences in weight, BUN, serum AST or serum ALT levels noted between these animals and comparably treated cotton rats given placebo. No significant histopathologic differences were seen in lungs of treated and placebo control animals.

Discussion

These studies were concerned primarily with the toxicity and antiviral activity of Cc3Ado against RSV and PIV3 in tissue culture and in cotton rats. The primary factors that motivated testing of Cc3Ado were the paucity of agents available for treating PIV3- and RSV-induced lower respiratory tract infections and reports that this and related compounds could inhibit paramyxoviruses in vitro (De Clercq and Montgomery, 1983; De Clercq et al., 1984).

Cc3Ado is a carbocyclic analogue of 3-deazaadenosine. It has been reported to act as a competitive inhibitor of S-adenosyl-L-homocysteine hydrolase, and because of this activity, to selectively inhibit methylation of the polynucleotide 5′ cap of viral mRNA, particularly the mRNA of negative (−)-stranded RNA viruses (Montgomery et al., 1982). This activity is reflected in reported ED_{50} and S.I. values for Cc3Ado in VERO cells of ≤ 0.4 μg/ml and ≥ 1000, respectively, against parainfluenza and measles viruses (De Clercq et al., 1984).

In the present studies, Cc3Ado was determined to have a mean ED_{50} value in HEp2 cells of 9 μg/ml (mean S.I.= 85) against RSV, and a mean ED_{50} against PIV3 of 14 μg/ml (mean S.I.= 55). The higher ED_{50} values obtained in the present studies could be due to the use of different cell lines, virus strains and/or multiplicities of infection.

Nonetheless, S.I. values ≥ 55 are noteworthy and as indicated by the data presented in Tables 2 and 3, Cc3Ado was efficacious in vivo against both viruses. The

mean minimum efficacious dose for Cc3Ado in cotton rats experimentally infected with RSV or PIV3 was 1 mg/kg/day, when given i.p., and 10 mg/kg/day when given orally by gavage. (Presumably, the pulmonary bioavailability of Cc3Ado was significantly reduced when given by gavage because of partial inactivation or poor transport of the drug from the stomach and/or the intestines.)

It is of interest to compare the antiviral activity of ribavirin and Cc3Ado in vitro and in vivo. In the present studies, the ED_{50} and S.I. values determined for ribavirin and Cc3Ado against RSV and PIV3 in tissue culture were comparable (Table 1). However, when given i.p. to cotton rats experimentally infected with RSV, the mean medium efficacious doses of Cc3Ado and ribavirin were somewhat disparate, 1 and 30 mg/kg/day, respectively (Table 2). The divergent activities of the two compounds in vivo may reflect differences between their transport, metabolism and compartmentalization in a whole animal.

Ribavirin has been shown to be considerably more active against RSV (Hruska et al., 1982) and influenza viruses (Wilson, et al., 1980) when given by small particle aerosol than when administered parenterally. In a study by Hruska et al. (1982) testing ribavirin against RSV in cotton rats, ED_{50} values of 200 and 4 mg/kg/day were obtained for ribavirin administered i.p. or by small particle aerosol, respectively. Other studies, performed using the mouse-influenza model, also suggest the superiority of aerosol-administered antiviral therapy over i.p. administration of drug for treating pulmonary virus infections (Stephen et al., 1977; Walker et al., 1976). We have not tested Cc3Ado by small particle aerosol since limited quantities of this compound were available for testing. Nonetheless, the data obtained in these studies show that when administered i.p. or orally, Cc3Ado is quite effective against RSV.

The fact that Cc3Ado is effective against RSV when given parenterally or orally is especially noteworthy. Small particle aerosol treatment of RSV with ribavirin is expensive and practical only for the treatment of severe RSV disease. There is a need to develop and license a compound to treat human RSV infection that can be given more practically and inexpensively. Cc3Ado given i.p. or orally may provide this opportunity.

Elucidation and licensing of a second compound with selective activity against members of the paramyxoviridae viruses may also permit use of combination therapy against these viruses. Combination therapy could increase the effectiveness of treatment and reduce the possibility of the development of resistant virus mutants. Studies on the effectiveness of ribavirin and Cc3Ado when used in combination against RSV are planned.

Using differences in weight, blood enzyme levels, overt symptomatology and pulmonary histopathology as parameters, no obvious toxic effects in cotton rats given Cc3Ado were observed in these tests, even in those animals administered daily and total doses well in excess of the minimal efficacious doses for this compound (20 mg/kg/day for eight days versus 1 or 10 mg/kg/day for three days). Although more extensive toxicity testing is desirable, the results of these studies are most encouraging and suggest that Cc3Ado has good selective activity against both RSV and PIV3.

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