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ANTIVIRAL EFFECTS OF SINGLE-STRANDED POLYNUCLEOTIDE INHIBITORS OF THE INFLUENZA VIRION-ASSOCIATED TRANSCRIPTASE AGAINST INFLUENZA VIRUS INFECTION OF HAMSTERS AND FERRETS

ELIZABETH M. ROUND and N. STEBBING*

Searle Research Laboratories, Lane End Road, High Wycombe, Bucks., U.K.

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Administration of a single-stranded polynucleotide copolymer containing 9% cytidine residues and 91% 4-thiouridine residues [poly(C,S^4U_{10})], a known potent inhibitor of the virion transcriptase of influenza viruses, suppressed the amount of virus recoverable from the nasal washes of influenza virus-infected hamsters and ferrets. The incidence of sneezing and nasal discharge in infected ferrets was also reduced. In hamsters, poly(C,S^4U_{10}) was more effective than amantadine-HCl given at a 5-fold higher dose; in ferrets, its effect was comparable with amantadine-HCl or Virazole. Polyinosinic acid in combination with poly-5-hydroxy cytidylic acid also had anti-influenza effects. Poly(C,S^4U_{10}) annealed to polyadenylic acid was not effective, nor was the double-stranded polymer (polyinosinic acid) · (polycytidylic acid), even when complexed with carboxymethylcellulose and polylysine. No toxic effects of poly(C,S^4U_{10}) were apparent in the treated hamsters and ferrets, and high doses ($\geqslant$ 2.86 g/kg) administered intraperitoneally to mice produced no adverse effects.

anti-influenza poly (C,S^4U_{10}) single-stranded polynucleotides influenza transcriptase
hamsters, ferrets poly(I)·poly(C)

INTRODUCTION

Although influenza viruses may be shown to be sensitive to interferon and interferon-inducing double-stranded polynucleotides in certain cell cultures [2, 4, 10] these agents have generally been found to have little effect against influenza virus infections in experimental animals. The polynucleotides were shown to be effective against lethal influenza virus infections in mice [7], but not against the non-lethal influenza infection in hamsters. In hamsters the agents induce little [6, 29] or no circulating interferon [9], and hamster cell cultures treated with the double-stranded polymer (polyinosinic acid) · (polycytidylic acid) [poly(I) · poly(C)] are not protected against infection [29]. The poor in vivo effect of interferon inducers against non-lethal influenza virus infections appears to be due to lack of response to interferon in vivo. Indeed, poly(I) · poly(C) effectively protects hamsters against lethal encephalomyocarditis (EMC) virus infection, and this effect

* To whom correspondence should be addressed, at his present address: Genentech, Inc., Research Labs., 460 Point San Bruno Blvd., So. San Francisco, CA 94080, U.S.A.

appears to be mediated by an interferon-like agent, although circulating interferon is not detectable [18].

In view of these observations we examined the effects, on influenza virus infection of hamsters and ferrets, of single-stranded polynucleotides known to inhibit the virion-associated transcriptase of influenza viruses [22]. This inhibition is base-specific: among a number of tested materials, polyuridylic acid [poly(U)] and a thiolated derivative, poly (C,S^4U_{10}), showed the most pronounced inhibition [22]. The inhibitory effect of polyribonucleotides is greater than that of polydeoxyribonucleotides, and inhibition only occurs if the polynucleotides are single-stranded [31]. In the present study, we compared the effects of single- and double-stranded polynucleotides on influenza virus infection of hamsters and ferrets.

MATERIAL AND METHODS

Viruses

Influenza A/Finland/74 (H_3N_2) was obtained from C. Sweet (University of Birmingham, U.K.). A/PR8/34 (H_0N_1) and A/Hong Kong/68 (H_3N_2) were obtained from J. Oxford (National Institute for Biological Standards, Hampstead, London, U.K.) and grown in 10 day embryonated eggs (Orchards Farm, Great Kingshill, Buckinghamshire, U.K.). Virus titrations were determined by the 'egg-bit' ($EBID_{50}$) assay [3], using a 50% end-point determined by the method of moving averages as described in Meynell and Meynell [11].

Chemicals

The single-standed polyribonucleotides, polycytidylic acid [poly(C)], polyinosinic acid [poly(I)], polyuridylic acid [poly(U)], polyadenylic acid [poly(A)], and the double-stranded complex, poly(I) · poly(C), and 4-thiouridine monophosphate were obtained from P-L Biochemicals (Milwaukee, WI). A copolymer containing 9% cytidine residues and 91% 4-thiouridine residues was prepared from poly(C) as described by Hochberg and Keren-Zur [5], and this material is here referred to as poly(C,S^4U_{10}). A copolymer containing 19% 5-bromocytidine residues and 81% 5-hydroxycytidine residues was prepared from poly(C) as described by Stebbing et al. [28], and for the sake of brevity this material is here referred to as poly(ho^5C). A nuclease-resistant complex of poly(I) · poly(C) and a colloid formed between carboxymethylcellulose and polylysine was prepared as described previously, and the same method was used for preparing a similar complex of poly(C,S^4U_{10}) [25]. Virazole was obtained from ICN Pharmaceuticals Inc. (Cleveland, OH) and amantadine-HCl from Sigma Chemical Co. (London, U.K.). Dilutions of virus stocks and polynucleotides were made in HBS buffer (0.89% (w/v) NaCl, 10 mM *N*-2-hydroxethyl-piperazine–*N'*-2-ethanesulfonic acid, pH 7.5).

Animals

Male Syrian golden hamsters (*Mesocricetus aureatus*), at 4 weeks of age and weighing 125–150 g, were obtained from Wrights Ltd. (Chelmsford, Essex, U.K.). Male ferrets (*Mustela furo*), weighing between 0.9 and 1.4 kg, were obtained from Froxfield Rabbits Ltd. (Petersfield, Hampshire, U.K.). Female BK:W (LACA) mice, for toxicity studies, were obtained from Bantin and Kingman Ltd. (Hull, U.K.) and used at 8 weeks of age when they weighed 21 g (± 1 g, range). Animals were maintained at 22°C with unlimited access to water. Hamsters and mice were given a diet of Rank–Hovis–McDougall's PMD cubes (Labsure, Poole, Dorset, U.K.) ad libitum, and ferrets were given 190 g of meat-based cat food ('Whiskas' from Pedigree Pet Foods, Melton Mowbray, Leistershire, U.K.) once daily at 9 a.m.

Hamsters were inoculated intranasally with 100 μl of virus (usually 10^3 $EBID_{50}$) influenza A/Hong Kong/68 after anaesthesia with Fluothane (Imperial Chemical Industries, Macclesfield, Cheshire, U.K.). Nasal washes were taken during the first 7 days post-infection and were obtained from conscious, restrained hamsters by gently introducing 50 μl of phosphate-buffered saline (PBS) into each nostril, alternately, until a total of 2.5 ml had been obtained. The nasal wash material produced during the procedure was collected in a Petri dish; 2% (w/v) bovine serum albumin (BSA, Sigma Chemical Co.) was added, and the samples were stored at -70°C until titrated.

Prior to infection ferrets were anaesthetised with sodium pentobarbitone (Saggatal, Abbot Labs., Queenborough, U.K.) and inoculated intranasally with 500 μl of virus (usually 10^3 $EBID_{50}$ influenza virus A/Finland/74). Nasal washes were taken daily for the first 7 days post-infection by the method of Potter et al. [14]. Ten ml nasal wash samples were obtained from conscious ferrets by instilling aliquots of ca. 0.4 ml PBS, pH 7.4, with a syringe into each nostril in turn, and collecting the expressed material. The samples were further processed as described above. For drug administration the ferrets were anaesthetised with pentobarbitone. Intravenous injections were given in the sublingual vein [12]; intranasal administrations were by inhalation.

Blood samples were removed by cardiac puncture before infection and 14 or 21 days after infection. Antibodies against influenza virus were determined by the haemagglutination inhibition assay described by Toms et al. [30].

Infection parameters

The following parameters of influenza virus infection of ferrets were determined.

V – the amount of virus detected in nasal washes taken once daily for the first 7 days post-infection averaged over the number of ferrets per group. Values given in the Figs. 1 and 2 are $EBID_{50}$/0.1 ml of nasal wash. In Table 1 the means of these determinations averaged over all the days of the experiment ($\bar{v}$) are given.

N – the amount of nasal discharge occuring at the morning feeding time, estimated as 0, 1, 2 or 3, according to severity; 0, no discharge; 1, watery discharge; 2, copious watery discharge; 3, thick mucopurulent discharge.

S – the incidence of sneezing occurring during the morning feeding time, estimated as 0 or 1, according to whether the ferret did or did not sneeze during the morning feeding and cleaning period (ca. 60 min). Values given in Table 1 are the sum of the sneezing indices over the first 10 days of infection, averaged for each group of ferrets. In Fig. 3, the percent sneezing values are calculated from the sneezing index of the infected control group, taken as 100%.

T – the rectal temperature determined daily at 9 a.m., using a clinical mercury thermometer for 7 days before infection and 10 days after infection.

Statistical analysis

The means ($\bar{v}$ and S) are tabulated. For $\bar{v}$ the significance of F values, calculated from the analyses of variance, are included and so also are 95% least significant difference (LSD) values, adjusted for the number of tests. The latter values can be used to compare any two $\bar{v}$ values within an experiment. Thus, if a particular difference between means is greater than the LSD, the means are significantly different at approximately the 5% level. The N, S and T data were summarized by summing over days to obtain a single total for each animal. For S, only the means and overall F values are given in Table 1. Since the distributional properties of these totals were not known, the Kruskal–Wallis non-parametric test for a one-way classification was used. After ranking the observations the H statistic is calculated [21]. H has a chi-squared distribution with $(k - 1)$ degrees of freedom, where k is the number of groups.

The differences between controls and poly(C,S^4U_{10})-treated ferrets were estimated for the data pooled on each day for seven experiments involving 25 control and 51 treated ferrets. For the $\bar{v}$ data it was important that treatment differences should be unrelated to variance and a graph of the control, poly(C,S^4U_{10}) difference versus the residual mean square confirmed this. Analysis of variance was used to assess significant differences in the $\bar{v}$ data. To correct for the different precision of the experiments a weighted analysis of variance was also carried out so as to reduce the emphasis of experiments having low group variance and more observations per group. Although S values showed variation between experiments, this parameter seemed amenable to summing over all experiments to obtain mean values for each day. Differences between control and poly(C,S^4U_{10})-treated groups were estimated by the Mantel–Haenszel test. The distribution of nasal discharge scores for all experiments on each day fitted a Poisson distribution and allowed direct comparison of the control and poly(C,S^4U_{10}) means on each day.

RESULTS

Polynucleotide treatment of influenza virus-infected hamsters

We found that inoculation of conscious hamsters as described by Renis [17] resulted in low levels of infection, probably because the hamsters are induced to sneeze, and we

therefore resorted to light Fluothane anaesthesia prior to infection. With this procedure as little as $5 \times 10^{2.5}$ $EBID_{50}$ of influenza A/Hong Kong/68 or A/Finland/74 caused all animals to be infected and to excrete in excess of 10^3 $EBID_{50}$/0.1 ml of nasal wash over several days post-infection. This dose of these viruses was therefore used routinely and a similar dose of A/PR8/34 (10^3 $EBID_{50}$) was found to cause comparable infection.

The effect of poly(C,S^4U_{10}) treatments on shedding of virus from the nasal passages of hamsters infected with A/PR8/34 and A/Finland/74 viruses is shown in Fig. 1. Single intraperitoneal (i.p.) treatments of 10 mg/kg 6 h before infection caused a non-significant reduction in virus shedding, but i.p. treatments of 10 mg/kg 6 h before infection and once daily for 6 days after infection caused decreased virus shedding. In the multiple treatment regimen, the first administration post-infection was at 18 h post-infection. The decrease in virus shedding was at least 10-fold with A/PR8/34 virus on days 2 and 3 post-infection and with A/Finland/74 virus on days 3, 4 and 5 post-infection. These reductions were statistically significant. Moreover, the total amount of virus shed, estimated by summing the amounts of virus detected over the 7 days of infection, was also significantly reduced with the multiple treatment regimen ($P < 0.01$). In comparison, amantadine-HCl treatments at 24 mg/kg daily for 7 days commencing with treatments 6 h before and 2 h after infection proved ineffective in reducing the amount of virus shedding.

These results demonstrated that poly(C,S^4U_{10}) administered as a single dose of 10 mg/kg 6 h before infection was not sufficient to decrease virus shedding from the nasal

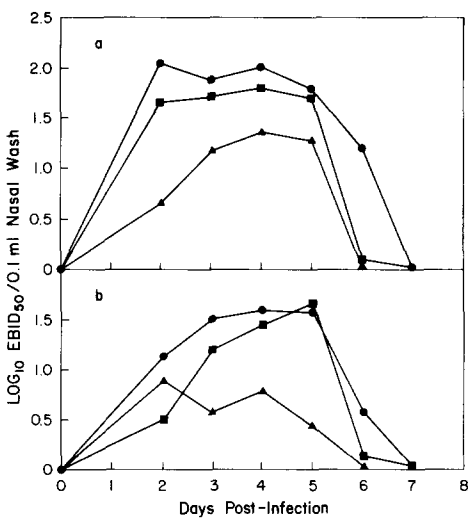


Fig. 1. The effect of poly(C,S^4U_{10}) administered intraperitoneally at 10 mg/kg each treatment on nasal virus shedding in hamsters injected with a) influenza virus A/PR8/34, and b) influenza virus A/Finland/74. ●, HBS-treated controls; ■, poly(C,S^4U_{10}) 6 h before infection; ▲, poly(C,S^4U_{10}) 6 h before infection and once daily on days 1, 2, 3, 4, 5 and 6 post-infection. Each point represents an average value over five hamsters.

passages of hamsters, whereas treatment with multiple doses was effective. Administration of the double-stranded complex, poly(I) · poly(C) was found to have no effect on influenza virus infection of hamsters, and multiple treatments with poly(C,S^4U_{10}) annealed to poly(A) [31] were also ineffective.

Polynucleotide treatments of influenza virus-infected ferrets

Intranasal inoculation of pentobarbitone-anaesthetised ferrets with 10^2, 10^3, 10^4 or 10^5 $EBID_{50}$ influenza virus (A/Finland/74) resulted in infection of increasing severity. At 10^2 $EBID_{50}$ not all animals were infected (1/6 showed no symptoms or virus shedding); at 10^5 $EBID_{50}$ symptoms were very severe and bleeding occurred from the nasal passages in all animals. Similar symptoms occurred in some but not all animals infected with 10^4 $EBID_{50}$. A virus dose of 10^3 $EBID_{50}$ was therefore used for routine tests on antiviral activity. With this dose virus was detected in nasal washes on days 1 and 2 post-infection; high virus titres were found between days 1 and 4 and virus became undetectable 6–8 days post-infection. The largest amount of virus detected in nasal washes of infected control ferrets varied between 2.5 and 4.5 $\log_{10}$ $EBID_{50}$/0.1 ml and occurred during the first 3 days post-infection. The total amount of nasal virus shedding averaged over number of days ($\bar{\nu}$) varied between 1.21 and 1.69 $\log_{10}$ $EBID_{50}$/0.1 ml.

Table 1 shows the results of experiments done to test the antiviral effect of poly(C,S^4U_{10}). Intraperitoneal as well as intranasal (i.n.) administration at 10 mg/kg/treatment once daily during the first 3 days of infection or over 5 days starting on day 1 before infection caused significant reduction in mean virus shedding. Intravenous (i.v.) administration did not cause a significant reduction, although the trend was for a reduction in this parameter, as well as in the sneezing score. The monomer, 4-thiouridine monophosphate, was ineffective and poly(U), which also inhibits the influenza virion transcriptase [22], likewise had no anti-influenza effects. The effect of poly(C,S^4U_{10}) was compared with that of other substances known to be effective against influenza or other virus infections. Amantadine-HCl (five i.p. injections at 50 mg/kg) caused a significant reduction in virus shedding comparable to that obtained with poly(C,S^4U_{10}) administered at 10 mg/kg per treatment by the intranasal route. Amantadine-HCl had no effect on nasal discharge but did affect the sneezing score to the same extent as poly(C,S^4U_{10}).

Toxic effects were observed after i.p. administrations of 50 mg/kg amantadine-HCl: for a few hours post injection ferrets showed pilerection, weakness and evidence of central nervous system effects but recovered within a few hours. The second experiment shown in Table 1 was done to compare the anti-influenza effects of poly(C,S^4U_{10}) with those of a mixture of poly(I) and poly(ho^5C), which is known to inhibit encephalomyocarditis virus infection of mice without inducing interferon [28]. Intraperitoneal administration of this mixture had significant effects on influenza virus infection of ferrets, although the component polymers were ineffective. In this experiment, intraperitoneal administration of poly(C,S^4U_{10}), at 10 mg/kg, three times over the first 48 h post-in-

TABLE 1

Comparison of the effects of various compounds on intranasal infection of ferrets with 10^3 $EBID_{50}$ influenza virus A/Finland/74

Treatment				Number of ferrets		Parameters of infection	
Administration route[a]	Compound	Dose (mg/kg each)	Time (h post-infection)			$\bar{\nu}$[b]	S[c]
i.p.	HBS	–	-24,0,24,48,72	6		1.69	5.7
i.p.	Amantadine-HCl	50	-24,0,24,48,72	6		1.37[d]	2.5
i.n.	Poly(C,S^4U_{10})	10	-24,0,24,48,72	6		1.18[d]	2.5
i.v.	Poly(C,S^4U_{10})	10	-24,0,24,48,72	6		1.54[f]	4.0
					F value LSD	0.3	d
i.p.	HBS	–	0,24,48	3		1.46	5.0
	Poly (C,S^4U_{10})	10	0,24,48	4		0.84[e]	2.8
	Poly(I)	10	0,24,48	4		1.39[f]	3.8
	Poly(ho^5C)	10	0,24,48	3		1.31[f]	2.8
	Poly(I), poly(ho^5C)	10	0,24,48	4		0.56[e]	4.0
					F value LSD	0.5	f

[a] i.p., Intraperitoneally; i.n., intranasally; i.v., intravenously.

[b] Viral shedding ($EBID_{50}$/0.1 ml of nasal wash; see Materials and Methods).

[c] Sneezing index (see Materials and Methods).

[d] P level for significance of difference with control <0.05.

[e] P level for significance of difference with control <0.001.

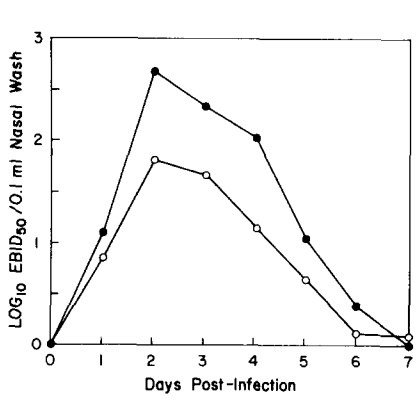
[f] Not significantly different from control.

fection, caused a significant reduction in virus shedding. However, the reduction in the sneezing score was not significant. In addition to the experiments listed in Table 1, we also included Virazole in our comparisons. It was found to decrease nasal virus shedding and also to reduce nasal discharge and sneezing. However, Virazole-treated ferrets ate less than half the amount of food consumed by control animals and lost 20% of their body weight over 7 days. This loss in body weight was also observed in Virazole-treated ferrets which were not infected.

In a set of seven experiments involving treatment with poly(C,S^4U_{10}) at 10 mg/kg per treatment, a consistent trend of reduced virus shedding, sneezing and nasal discharge was observed, although these parameters were not all significantly affected in all experiments. There were no apparent qualitative differences in the effects of poly(C,S^4U_{10}) treatments given by different routes or for various time periods (once or twice daily for 3 days or for 3 or 5 days). Therefore the data of all seven experiments were pooled. It was reasoned that this should not result in any particular bias, because the change in mean effects would be counterbalanced by the increase in variation which is taken into account in assessment of significance levels. The data were pooled for each of the first 10 days post-infection. Variance analyses failed to reveal interactions between treatments and days post-infection, implying that poly(C,S^4U_{10}) did not affect the time course of virus shedding. Fig. 2 shows that poly(C,S^4U_{10}) reduced virus shedding. This was statistically significant whether or not the contributing means were weighed by experimental error of the mean values from different experiments. When totalled over the seven experiments, the incidence of sneezing showed a biphasic trend with a peak on day 3 post-infection and a second elevation from day 6 onward (Fig. 3a). There was no evidence for a significant effect of poly(C,S^4U_{10}) treatment on the initial peak, but there was a significant reduction of about 45% on each day in the second phase (days 6–10). The overall decrease in sneezing was statistically significant ($P < 0.025$). Mean nasal discharge for control groups also showed a biphasic trend with peaks at days 2 and 7 (Fig. 3b). Poly (C,S^4U_{10}) treatment caused a reduction and delay by one day in the first peak and a significant reduction by about 50% during both the first and the second phases (Fig. 3b). Infection caused a significant increase of body temperature. Although a smaller increase was consistently observed in poly(C,S^4U_{10})-treated than in untreated ferrets, this was not statistically significant.

We examined the anti-influenza effect of poly(I) · poly(C) in several experiments and found that administration of 4 mg/kg 24 h before infection or 24 h before infection and once daily for 2 days after infection had no significant effects on any parameter of infection. Because of the possibility that poly(I) · poly(C) was degraded by ferret serum nucleases as it is in primate serum [8], we examined the effect of poly(I) · poly(C) complexed with carboxymethylcellulose and polylysine. In primates, this complex is a potent interferon inducer and provides protection against experimental infection [8]. Yet it proved ineffective against influenza virus infection in ferrets.

The titre of antibodies against influenza virus was determined in all the experiments at 14 and/or 21 days post-infection. With the exception of Virazole, which is known to



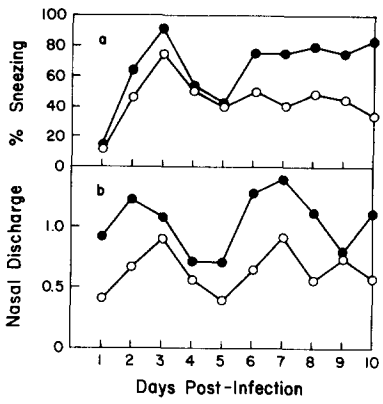


Fig. 2. The effect of poly(C,S^4U_{10}) treatment (10 mg/kg/day during the first 3 days after infection) on virus shedding in ferrets injected with influenza virus A/Finland/74 (10^3 $EBID_{50}$: ○), as compared with control (HBS-treated: ●) ferrets. Values are means from seven experiments in which different administration routes were used (see text; n = 25 for HBS control group and n = 51 for poly(C,S^4U_{10})-treated group).

Fig. 3. The effect of poly(C,S^4U_{10}) treatment on a) percent sneezing, and b) mean nasal discharge in influenza virus A/Finland/74-infected ferrets. See legend to Fig. 2. ○, treated group; ●, control group.

be immunosuppressive [13], none of the treatments investigated caused any change in the antibody titres, which ranged from 1280 to 2560.

Toxicity of poly(C,S^4U_{10})

No toxic effects attributable to the treatments were observed in hamsters or ferrets during the antiviral studies with poly(C,S^4U_{10}). Insufficient poly(C,S^4U_{10}) was available to assess toxicity of high doses in uninfected hamsters or ferrets, but we did investigate acute toxicity in mice. Intraperitoneal administration of 10, 20, 40 or 60 mg/mouse to groups of 10 mice produced no deaths or abnormal effects during a 14 day interval post-treatment. In contrast, poly(I) · poly(C) had an LD_{50} of 48 mg/kg and caused obvious pilerection, diarrhoea and polyuria at 36 mg/kg. The highest dose of poly(C,S^4U_{10}) tested in this toxicity study was 2.86 g/kg.

DISCUSSION

The potent double-stranded polynucleotide interferon inducer, poly(I) · poly(C), proved to have no effect on parameters of influenza infection of hamsters or ferrets, and this would not seem to be due to high serum nuclease concentrations in ferrets because poly(I) · poly(C) was no more effective when complexed with carboxymethylcellulose–polylysine, which is known to render poly(I) · poly(C) effective in primates

[8]. Poly(C,S^4U_{10}) was optimally effective when administered at and after the time of infection and was ineffective when administered before infection. These observations imply that poly(C,S^4U_{10}) does not exert a direct virucidal effect on the parental virus inoculum and that interferon induction does not occur. Unlike double-stranded polynucleotides, single-stranded polynucleotides do not seem to act as immune adjuvants [19], and modulation of immune responses therefore seems an unlikely mode of action. The observation that poly(C,S^4U_{10}) protects influenza virus-infected infant rats against bacteraemia arising from subsequent *Haemophilus influenzae* infection [16] indicates that poly(C,S^4U_{10}) protects the nasal epithelium from destruction by influenza virus infection and is in agreement with the present antiviral effects.

Poly(C,S^4U_{10}) causes 50% inhibition of the in vitro activity of the virion-associated transcriptase of influenza viruses at a concentration 4 μg/ml or less [22]. The immediate blood concentration achievable in ferrets after intravenous administration of 10 mg/kg of poly(C,S^4U_{10}) is about 20 times this concentration. An intracellular concentration of poly(C,S^4U_{10}) sufficient to inhibit substantially the influenza transcriptase is therefore possible. Polynucleotides do appear to be incorporated by cells in vitro and in vivo, although the mechanisms mediating in vivo accumulation are obscure [23].

It is noteworthy that anti-influenza effects of poly(C,S^4U_{10}) are only apparent when multiple treatment times are used, including a treatment close to the time of infection. In contrast, significant antiviral effects against other virus infections conferred by single-stranded polynucleotides may occur with single treatments at 6 h before infection [24–26, 28]. However, the anti-influenza effects of poly(C,S^4U_{10}) reported here are encouraging, because treatment of influenza virus infection of hamsters and ferrets appears to be relevant to the human disease [13] and the antiviral effects are comparable to those of amantadine–HCl and Virazole [15, 20]. Moreover, the toxic effects of amantadine-HCl and Virazole have not been observed with poly(C,S^4U_{10}) treatments, and a range of influenza viruses are affected by poly(C,S^4U_{10}) (A/PR8/34; A/Hong Kong/68; A/Finland/74). The intranasal route, considered ineffective for other agents [1] seems, in the case of poly(C,S^4U_{10}), to be at least as effective as intraperitoneal treatments. The absence of consistent and statistically significant effects on body temperature with poly (C,S^4U_{10}) and other agents has been considered elsewhere [27].

The present results are consistent with the hypothesis that the anti-influenza effects of poly(C,S^4U_{10}) are, in part at least, mediated by inhibition of the virion transcriptase and not by induction of interferon or stimulation of long-term antibody responses. However, there are several alternative methods by which poly(C,S^4U_{10}) could inhibit influenza virus infection. Poly(S^4U) binds to rat liver ribosomes and causes inhibition of protein synthesis [5]. This effect is about 20 times more pronounced for exogenous synthetic polynucleotide-stimulated protein synthesis than for endogenous protein synthesis, and possibly influenza mRNAs in vivo respond in the same way as exogenously added mRNAs in the cell-free system. However, the low toxicity of poly(C,S^4U_{10}) is noteworthy even when compared with other single-stranded polynucleotide treatments [26]. Other possible antiviral mechanisms have been discussed elsewhere in relation to antiviral

effects of single-stranded polynucleotides against picorna- and arbo-virus infections [24]. Stimulation of cellular immune responses also remains a possible mode of action for poly(C,S^4U_{10}) against influenza virus infection, and this possibility is under investigation.

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REFERENCES

1 Buncknall, R.A. (1976) in: Chemotherapy and Control of Influenza, eds. J.S. Oxford and J.D. Williams (Academic Press, London).
2 Burke, D.C. and Isaacs, A. (1958) Br. J. Exp. Pathol. 34, 452.
3 Fazekas De St. Groth, S. and White, D.O. (1958) J. Hyg. (London) 56, 151.
4 Hill, D.A., Baron, S. and Chanock, R.M. (1969) Lancet 2, 187.
5 Hochberg, A.A. and Keren-Zur, M. (1974) Nucleic Acids Res. 1, 1619.
6 Jahrling, P.B., Navarro, E. and Scherer, W.F. (1976) Arch. Virol. 51, 23.
7 Kleinschmidt, W.J. and Streightoff, F. (1971) Infect. Immun. 4, 738.
8 Levy, H.B., Baer, G., Baron, S., Buckler, C.E., Gibbs, C.J., Iadarola, M.J., London, W.T. and Rice, J. (1975) J. Infect. Dis. 132, 434.
9 McNeil, T.A., Flemming, W.A. and McLance, D.J. (1972) Immunology 22, 711.
10 Merigan, T.C., Hall, T.S., Reed, S.E. and Tyrrell, D.A.J. (1973) Lancet 1, 563.
11 Meynell, G.G. and Meynell E. (1965) Theory and Practice in Experimental Bacteriology (Cambridge University Press).
12 Moran, M. (1979) J. Inst. Animal Techn. 30, 45.
13 Potter, C.W. and Oxford, J.S. (1977) in: Chemoprophylaxis and Virus Infections of the Respiratory Tract. Vol. 2, ed. J.S. Oxford (CRC Press, Cleveland, OH) p. 1.
14 Potter, C.W., Oxford, J.S., Shore, S.L., McLaren, C. and Stuart-Harris, C. (1972) Br. J. Exp. Pathol. 53, 153.
15 Potter, C.W. and Schofield, K.P. (1975) in: Symmetrel in Virology, ed. C.A.S Wink (Geigy Pharmaceuticals, Macclesfield, U.K.) p. 24.
16 Potter, C.W., Teh, C.Z., Harvey, L. and Jennings, R. (1980) Antimicrob. Agents Chemother. 7, 575.
17 Renis, H.E. (1977) Arch. Virol. 54, 85.
18 Round, E.M. and Stebbing, N. (1981) J. Interferon Res. (in press).
19 Schmidtke, J.R. and Johnson, A.G.(1971) J. Immunol. 106, 1191.
20 Schofield, K.P., Potter, C.W., Edey, D., Jennings, R. and Oxford, J.S. (1976) in: Chemotherapy and Control of Influenza, eds. J.S. Oxford and J.D. Williams (Academic Press, London) p. 63.
21 Siegel, S. (1956) Nonparametric Statistics for the Behavioral Sciences (McGraw-Hill).
22 Smith, J.C., Raper, R.H., Bell, L.D., Stebbing, N. and McGeoch, D. (1980) Virology 103, 245.
23 Stebbing, N. (1979) Cell Biol. Int. Rep. 3, 485.
24 Stebbing, N. (1979) Pharmacol. Ther. A 6, 291.
25 Stebbing, N. and Dawson, K.M. (1979) Mol. Pharmacol. 16, 313.
26 Stebbing, N. and Grantham, C.A. (1976) Arch. Virol. 51, 199.

27 Stebbing, N., Healey, G. and Round, E. (1981) J. Virol. Methods.
28 Stebbing, N., Lindley, I.J.D. and Eaton, M.A.W. (1977) Proc. R. Soc. London Ser. B 198, 411.
29 Stewart, W.E., Scott, W.D. and Sulkin, S.E. (1969) J. Virol. 4, 147.
30 Toms, G.C., Bird, R.A., Kingsman, S.M., Sweet, C. and Smith, H. (1976) Br. J. Exp. Pathol. 57, 37.
31 Weck, P., Jackson, M., Stebbing, N. and Raper, R. (1980) in: 4th Negative Strand Virus meeting.