Effect of Poly I: C on Experimental Respiratory Infection in Hamsters

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The interferon inducer double-stranded polyinosinic acid and polycytidylic acid (poly I: C) was studied in hamsters experimentally infected with parainfluenza 3 virus. Upper intranasal, deep intranasal, or intraperitoneal treatment of hamsters with poly I: C (100 µg/100- to 120-g animal) 24 hr before an upper respiratory infection significantly reduced the virus titer in the lungs, as measured 48 hr after a deep lung infection with parainfluenza 3 virus; however, the upper respiratory poly I: C treatment was ineffective.

Double-stranded complexes of polyinosinic acid and polycytidylic acid (poly I: C) have been shown to be highly active in the induction of interferon in vitro and in vivo (4). The antiviral activity of poly I: C has been studied in several experimental respiratory infections in mice, where it was found that poly I: C was highly protective against pneumonia virus of mice and parainfluenza 1 infections, moderately active against influenza B infections, and marginally active against infections with influenza A, A-1, or A-2 viruses (4, 7, 9). Cell cultures pretreated with poly I: C were shown to be protected from destruction by several clinically important respiratory viruses, including influenza A-2, respiratory syncytial virus, parainfluenza 1, and rhinovirus 13 (6).

Experimental lung infections of hamsters with parainfluenza 3 virus have been described (1, 2, 8). Furthermore, it has been shown that, under certain conditions, an infection of the turbinates can be achieved which can be used as a model of an upper respiratory infection (13). By using this model, virus titers in the nose washes were reduced when hamsters were treated with nose drops containing certain α-ketoaldehydes, agents with virucidal and virustatic properties (H. E. Renis, 6th Int. Congr. Chemother. Abstr., 1969), and calcium elenolate, a virucidal agent (12, 14).

Poly I: C has been shown to induce interferon in hamsters (15). The study reported herein was undertaken to determine whether an interferon inducer (poly I: C) had any effect on an infection of turbinates or lungs in hamsters with parainfluenza 3 virus.

MATERIALS AND METHODS

Poly nucleotides. Poly I and poly C were obtained from Miles Laboratories, Inc., Elkhart, Ind., or P-L Biochemicals, Inc. Poly I: C was prepared in these laboratories (4) or purchased from P-L Biochemicals, Inc.

Virus. Myxovirus parainfluenza type 3 (HA-1 virus, strain C-243) was obtained from the American Type Culture Collection. The virus was propagated in ML monolayers (obtained from D. A. Buthala) in medium 1066 with 0.5% inactivated fetal bovine serum. The virus titers were determined after diluting the samples in Hanks balanced salt solution. Monolayers of Hep-2 cells in 60-mm plastic petri plates (Falcon Plastic, Div. of B-D Laboratories, Inc., Los Angeles, Calif.) were washed with Hanks solution and infected by adding 0.5 ml of the virus. After 60 min at 37°C to allow for virus attachment, 4 ml of medium 199 containing 5% inactivated fetal bovine serum and 1% agar (Noble) was added to each plate, and the plates were returned to the incubator. The plaques were counted 48 hr later after staining with neutral red.

Hamsters. Male Syrian Golden hamsters (100 to 120 g) were purchased from Lakeview Hamster Colony, Newfield, N.J. The animals were used within 1 week after arrival. During the experiment the animals were housed in steel wire-mesh cages with five animals in each cage.

Animal handling. The method described by Soret (13) was used in studies involving parainfluenza 3 virus infection of the nasal cavity. The methods of virus inoculation and obtaining nasal washes were as described. The virus was delivered in 0.01 ml into one nostril and contained about 7 × 10⁶ plaque-forming units (PFU). Nose washes were taken at 24 to 28 hr postinfection, and the virus titers were determined by the plaque method on Hep-2 monolayers.

Infection of the lungs of hamsters with parainfluenza 3 virus was described (1, 2, 8). In these studies,
parainfluenza 3 virus with about $7 \times 10^8$ PFU in 0.1 ml was given to hamsters after light carbon dioxide anesthesia. At 48 hr after infection, the animals were anesthetized with 0.5 ml of 1:30 pentobarbital in saline by intraperitoneal (IP) injection. The lungs were removed surgically and stored at $-40$ C. At the time of assay for virus, each lung was homogenized in a Potter-type homogenizer in 3 ml of saline containing 0.01 M phosphate buffer (pH 7.5). The homogenates were clarified by centrifugation and were diluted in Hanks solution. Duplicate Hep-2 monolayers were inoculated with each dilution of virus, and the virus titers were determined by the plaque method.

For statistical analysis, the virus titer (PFU/ml) obtained from each animal was converted to logarithms. The mean value obtained for each drug-treated group was compared with the corresponding control. The significance of the observed difference was determined by the Student $t$ test. Dunnett’s (3) test was used when more than one treatment group was compared to the control group.

**Drug treatment.** The hamsters were treated with the polynucleotides by three routes: (i) upper intranasal (IN), with 0.05 ml of the preparation given into each nostril of the animal without anesthesia; (ii) deep IN, with 0.1 ml of the preparation given intranasally to the animals after light carbon dioxide anesthesia to allow the drug to enter the lungs; and (iii) IP, with 0.1 ml of the preparation injected into the peritoneal cavity of the animal. In the experiments reported here, single doses of the polynucleotides were usually given 24 hr before infection.

**RESULTS**

The effect of treating hamsters with poly I:C at 24 hr before infecting the nasal cavity with parainfluenza 3 virus is shown in Table 1. In this experiment, poly I and poly C were included as controls. The polynucleotides were given by two routes: (i) upper IN, i.e., 0.05 ml of each material was placed in each nostril without anesthetizing the animal, and (ii) IP, virus titers in each of the nasal washes of the 10 animals comprising each treatment group are given. Neither poly I nor poly C was effective in reducing the virus titers whether given IN or IP. However, poly I:C caused a significant reduction in virus titers when given by either the IN or the IP route. These data show that poly I:C, but not the homopolymers, was effective in reducing the virus yields as recovered in the nasal washes from hamsters infected with parainfluenza 3 virus in the nasal cavity.

The experiment shown in Table 2 was carried out to confirm the effect of poly I:C on the upper respiratory infection and to determine what effect deep IN treatment with poly I:C might have on the virus yields. The data indicate that the virus yields are significantly reduced from those animals which have been treated with poly I:C by upper IN, deep IN, or IP treatment. Whether the effects seen with the deep IN treatment reflect the interferon formation in the lung which is transported to the nasal cavity, or if the effects seen with deep IN treatment simply indicate that the poly I:C adsorsbs to the tissue of the nasal cavity on its way to the lung, is not known. However, these data clearly show that poly I:C pretreatment by all routes is effective in reducing the virus yields in the nasal washes.

The effect of pretreating hamsters with poly I:C on parainfluenza 3 virus infections in the lungs of the animals is shown in Table 3. In this experiment, as in that described in Table 2, the animals were treated with the polynucleotides by upper IN, deep IN, or IP. The data show that the upper IN treatment was ineffective in reducing the virus yields, whereas both the deep IN and the IP treatments were effective. These data, taken with those in Table 2, suggest that IP or

### Table 1. Parainfluenza 3 virus titers (PFU/ml) in nose washes of hamsters pretreated with polynucleotides

| Treatment | Virus titers |
|-----------|--------------|
|           | Poly I | Poly C | Poly I:C | Saline |
| **IN**    |        |        |         |        |
| 1         | 349    | 183    | 5       | 246    |
| 2         | 3,900  | 256    | 1       | 315    |
| 3         | 72     | 2,900  | 126     | 503    |
| 4         | 113    | 20,000 | 307     | 136    |
| 5         | 2,900  | 105    | 41      | 76     |
| 6         | 778    | 240    | 10      | 2,300  |
| 7         | 786    | 4,600  | 34      | 119    |
| 8         | 65     | 245    | 141     | 21     |
| 9         | 3,700  | 244    | 15      | 36     |
| 10        | 25,000 | 144    | 31      | 458    |
| Mean      | 2.91   | 2.74   | 1.43b   | 2.26   |
| Δ log (log) | 0.65  | 0.48   | -0.83   |        |

| **IP**    |        |        |         |        |
| 1         | 11,200 | 18,800 | 20      | 628    |
| 2         | 13,900 | 814    | 67      | 352    |
| 3         | 5,800  | 3,700  | 79      | 2,100  |
| 4         | 26     | 92     | 200     | 2,400  |
| 5         | 273    | 103    | 26      | 34     |
| 6         | 2,100  | 112    | 4       | 1      |
| 7         | 3,200  | 634    | 1       | 976    |
| 8         | 236    | 17,000 | 6       | 1,800  |
| 9         | 139    | 239    | 6       | 3,500  |
| 10        | 4,000  | 390    | 2       | 112    |
| Mean      | 3.08   | 2.89   | 1.20c   | 2.54   |
| Δ log (log) | 0.53  | 0.34   | -1.35   |        |

* a Standard deviation for IN treatment was 0.74, and for IP treatment it was 0.90.
* b $P < 0.05$.
* c $P < 0.01$.
deep IN treatment protects both the upper and lower respiratory tract of the animals from virus infection. Upper IN treatment protects only the upper nasal cavity, and in this sense has a "topical" effect. IP treatment probably involves a humoral interferon and thus protects the entire organism.

**Table 2. Parainfluenza 3 virus yields (PFU/ml) in nose washes from hamsters treated with poly I:C by different routes**

| Route | IN (upper)a | IN (deep)b | IPc |
|-------|-------------|-------------|-----|
|       | Poly I:C    | Saline      | Poly I:C | Saline | Poly I:C | Saline |
| 1     | 12          | 34          | 60      | 200    | 8         | 258    |
| 2     | 3           | 415         | 73      | 60     | 2         | 298    |
| 3     | 17          | 144         | 4       | 153    | 1         | 890    |
| 4     | 33          | 212         | 64      | 171    | 1         | 176    |
| 5     | 1           | 158         | 36      | 322    | 1         | 127    |
| 6     | 40          | 138         | 1       | 90     | 3         | 585    |
| 7     | 8           | 382         | 1       | 1,016  | 7         | 636    |
| 8     | 8           | 169         | 1       | 156    | 1         | 96     |
| 9     | 39          | 193         | 22      | 191    | 4         | 54     |
| 10    | 7           | 41          | 2       | 84     | 3         | 297    |
| Mean (log) | 1.05 | 2.17 | 0.95 | 2.24 | 0.45 | 2.40 |

a Change in the log for upper IN was -1.12, standard deviation was 0.43, and P was <0.01.

b Change in the log for deep IN was -1.28, standard deviation was 0.62, and P was <0.01.

c Change in the log for IP was -1.94, standard deviation was 0.37, and P was <0.01.

**Table 3. Parainfluenza 3 virus titers (PFU/ml) in lung homogenates from hamsters treated with poly I:C by different routes**

| Hamster no. | Treatment route |
|-------------|-----------------|
|             | Poly I:C | Saline | Poly I:C | Saline | Poly I:C | Saline |
| 1           | 46,500 | 35,000 | 420     | 30,200 | 260     | 39,600 |
| 2           | 31,600 | 6,300  | 10      | 37,000 | 420     | 104,000|
| 3           | 28,200 | 25,000 | 10      | 66,400 | 60      | 16,500 |
| 4           | 41,200 | 25,600 | 250     | 42,000 | 220     | 24,600 |
| 5           | 8,340  | 4,400  | 430     | 25,000 | 80      | 24,000 |
| 6           | 54,200 | 14,200 | 1,620   | 350    | 1,660   | 67,000 |
| 7           | 2,710  | 6,700  | 80      | 49,000 | 490     | 53,000 |
| 8           | 26,800 | 50,800 | 490     | 78,000 | 5,280   | 33,600 |
| 9           | 38,000 | 19,600 | 10      | 12,400 | 330     | 49,300 |
| 10          | 22,300 | 39,400 | 10      | 51,000 | 500     | 182,000|
| Mean (log)  | 4.37   | 4.24   | 1.96    | 4.38   | 2.59    | 4.66   |

a Change in the log for upper IN was 0.13, standard deviation was 0.38, and P was nonsignificant.

b Change in the log for deep IN was -2.4, standard deviation was 0.77, and P was <0.01.

c Change in the log for IP was -2.07, standard deviation was 0.46, and P was <.01.

**DISCUSSION**

The available literature indicates that interferon and several interferon inducers inhibit the multiplication of a broad spectrum of respiratory viruses in pretreated cell cultures (6, 16; Klein- schmidt, Abstr., 2nd Conf. Antiviral Substances, N.Y. Acad. Sci., 1969). In vivo experiments with statolon, an interferon inducer of mold origin, showed that, unless the inducer is given by the IN route, it is ineffective in protecting mice from the lethal effects of influenza infection (10, 11). Interferon itself has shown variable results on experimental influenza infections in mice. Takano et al. (16) were able to protect mice with interferon pretreatment, whereas Finter (5) failed to demonstrate any effect on the growth rate of influenza virus in the lungs of mice even though high doses of interferon had been given 18 to 24 hr before infection. Although different routes of interferon treatment were used (intravenous, intramuscular, or IP), the IN route was not used. It was shown that mouse trachea organ cultures which had been pretreated with interferon and subsequently infected with influenza virus yielded less virus initially than untreated cultures. The problems inherent in treating animals by the IN route have been adequately outlined (5).

The studies reported herein clearly demonstrate that pretreatment of 100- to 120-g hamsters with 100 μg of poly I:C by upper IN, deep IN, or IP routes resulted in decreased virus yields from an upper respiratory infection with parainfluenza 3 virus. Although enough of the inducer may have
adsorbed to the tissues in the nasal cavity during the deep IN treatment to allow for local protection, the observation that the IP treatment route also protected the nasal cavity is suggestive evidence that an inhibitor is formed which protects cells at a distant site. Stewart et al. (15) showed that interferon could be demonstrated in the serum of weanling hamsters 6 hr after an IP injection of 100 μg of poly I:C. However, pretreatment of hamster kidney monolayers with 20 μg of poly I:C per ml did not confer resistance against any of several challenge viruses, probably because of the lack of uptake by the poly I:C. Their studies showed that viruses have a wide variation in susceptibility to poly I:C-induced resistance, and cell systems varied in response to poly I:C pretreatment.

By contrast, these studies indicate that reduced virus yields in the lungs of hamsters which had been infected with parainfluenza 3 virus after anesthesia resulted only when the hamsters had been pretreated with poly I:C by the deep IN or IP routes but not by the upper IN routes. Again, the formation of an inhibitor which acts at a distant site is implicated in case of the IP treatment. Whether the deep IN treatment represents a strictly local protection is not known. However, it has been found that IP treatment with poly I:C protected hamsters from a lethal IN challenge of encephalomyocarditis virus, whereas the IN poly I:C (given either upper or deep) was without effect (H. E. Renis, unpublished data). Thus, it appears that, under the conditions of upper IN pretreatment with poly I:C, the cells of the upper respiratory tract became resistant to virus infection, but those of the lung remained fully susceptible. IP treatment probably resulted in circulating interferon which essentially protected the entire organism from virus infection.

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