Effects of Oxyamylose and Polyacrylic Acid on Foot-and-Mouth Disease and Hog Cholera Virus Infections

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Two interferon-inducing polycarboxylates were tested for antiviral activity on foot-and-mouth disease (FMD) virus infections in mice, guinea pigs, and swine. Polyacrylic acid, given intraperitoneally, had a protective effect on infection by FMD virus administered in the peritoneal cavity of mice and in the foot pad of guinea pigs. Chlorite-oxidized oxyamylose (COAM) was effective in mice at a dosage of 2 mg/kg. Swine were not protected against naturally transmitted FMD by 120 mg/kg of COAM nor by polyacrylic acid. Swine were not totally unresponsive to COAM since it delayed symptoms of hog cholera. Interferon was not detected in the serum of COAM-treated swine. With FMD virus, an example was found of activity of interferon inducers in experimental hosts and lack of activity in a natural host.

Synthetic polycarboxylic acids, or carboxylate polymers, are a new class of antiviral compounds which induce transient levels of interferon in the blood of the intact animals. They protect it against the effects of a virus challenge administered up to many weeks later. Pyran copolymer (14), polyacrylic acid (6, 9, 10), and chlorite-oxidized oxyamylose (COAM; 2, 4) have been studied in detail.

These compounds appear to offer particular interest for the prophylaxis against viral disease in veterinary medicine. As the complex of polyniosinic and polycytidylic acids (poly I:C; 12, 15) and other antiviral ribonucleic acid (RNA) species, they are much more active when given before exposure than for cure of viral infections. Unlike the antiviral RNA species, they display prolonged antiviral activity. An acceptable compound for prophylaxis in animals will probably belong to the long-acting group of interferon inducers, such as the carboxylate polymers, rather than to the short-acting group, such as poly I:C. Furthermore, experience with the long-acting compounds in naturally occurring viral disease of large animals could provide more realistic information with regard to their possible application in man than may be derived from experimental infection of small laboratory animals.

The broad host range of foot-and-mouth disease (FMD) virus and its importance in natural hosts (1) seem to make FMD virus infection an appropriate model for testing the activity of interferon-inducing polycarboxylates. The purpose of this study was to ascertain whether polyacrylic acid and COAM have a protective effect in experimental rodents and in swine, a natural host. Polyacrylic acid was found to be effective in mice and in guinea pigs but not in swine. Similar findings were obtained in mice and in swine with COAM, a highly active, potentially more useful polycarboxylate which became available more recently (2, 4). The results of this investigation also led us to examine interferon induction and protection against hog cholera in swine by COAM.

MATERIALS AND METHODS

Animals. Conventional guinea pigs and swine were used. Pregnant pathogen-free CFLP mice were obtained from Carworth Europe Laboratories, Huntingdon, England.

Viruses. FMD virus, type 0, Vienna, was obtained from G. Kubin, Vienna, Austria. It was passaged three times in cattle and four times in guinea pigs, from the lesions of which it was harvested for use in the experiments on guinea pigs. FMD virus, type 0, Lausanne, was obtained from G. Moosbrugger, Basle, Switzerland, and passaged once in cattle. Then it was passaged three times in suckling mice and harvested from infected muscles for use in the experiments on mice. Another sample was passaged three times in swine, and the virus was harvested from the lesions for use in the experiments on swine. With the adaptive passages,
the type 0, provoked early lesions, appearing nearly simultaneously in all infected guinea pigs and swine, and rapid death in mice infected under 10 days of age. Unlike mice, guinea pigs and swine generally recovered from FMD virus infection.

The Behring 65 strain of hog cholera virus was obtained from Behring Laboratories, Marburg, West Germany, and passaged three times in swine. The virus stock was defibrinated viremic blood. All swine succumbed to infection with this virus.

Swine interferon and FMD virus antibody assays. The continuous porcine kidney cell line IB-RS-2 (5) and primary swine kidney cells were grown and used for assay of swine interferon as described for human and rabbit cells and interferons (7). The methods were plaque reduction and yield reduction of vesicular stomatitis virus (VSV). Neutralizing antibody to FMD virus was assayed by mixing equal volumes of doubling dilutions of swine serum and 100 median tissue culture infective doses of swine-adapted FMD virus, as mentioned above. The mixtures were kept for 1 hr at 37 C. End points of neutralizing antibody activity were measured by the suppression of FMD virus cytopathic effect in IB-RS-2 cells.

Antiviral compounds. Polyacrylic acid and COAM were prepared as previously described (4, 9). They induced interferon in mice (4, 10) and protected mice against viral infection as described (2, 6). The polymers were dissolved at appropriate concentrations in phosphate-buffered saline and injected intraperitoneally in volumes of 1 ml in guinea pigs, 0.1 ml in sucking mice, and at concentrations of 10 or 30 mg/ml in swine. For subcutaneous injection of COAM in swine, a concentration of 30 mg/ml was used.

Statistically significant delay of mortality in mice infected with FMD virus was taken as an index of antiviral activity, rather than indefinite survival. Although indefinite survival was observed in treated mice, it was a less sensitive criterion of antiviral activity. Similarly and unless otherwise noticed, the criterion for protective activity in guinea pigs and in swine was delay in the appearance of lesions.

RESULTS

FMD virus infection and polyacrylic acid. Suckling mice, 5 to 7 days of age and weighing 4 or 5 g, were injected intraperitoneally with 20 mg of polyacrylic acid per kg. This dosage did not cause mortality, growth inhibition, or other apparent ill effects during an observation period of 3 weeks. Eighteen hours after injection of the compound, FMD virus was administered through the same route. With the higher virus inocula, mice started succumbing on the second day after virus administration. Older mice could not be used because of their resistance to the virus. In all experiments, mortality due to FMD virus was delayed in polyacrylic acid-treated mice. The protective effect of 20 mg of polyacrylic acid per kg against different virus challenge doses is recorded in Fig. 1. Results obtained from three litters of eight mice have been pooled. One LDX0 was the virus dose which killed half of the mice after 5 days, as measured in preliminary experiments. Mortality was delayed with virus challenge doses as high as 10,000 LD50.

Protection experiments were also performed in guinea pigs. These animals did not die after virus administration in the planter pad, but they did develop typical lesions as seen in the natural hosts. The guinea pig model provides the best approximation in small animals of the infection that develops in naturally susceptible large animals (1). Guinea pigs weighing 500 g were injected intraperitoneally with 5 or 20 mg of polyacrylic acid per kg. After 18 hr, FMD virus was administered in the plantar pad. Both dosages of the compound were found to protect the animals against generalization of the infection. A representative experiment is shown in Table 1. The appearance of lesions at the injection site was also delayed. However, a number of guinea pigs died as a result of the toxicity in this animal of protective doses of polyacrylic acid.

Swine were chosen to test the activity of polyacrylic acid against naturally transmitted FMD in a naturally susceptible host. Twelve pigs weighing between 16 and 20 kg were given 30 mg of polyacrylic acid per kg intraperitoneally. Six were exposed after 18 hr and six after 7 days to swine having FMD. All treated swine developed symptoms at about the same time as the untreated, simultaneously exposed, control group. Three additional swine treated 3 days before exposure also showed no effect. The dosage of polyacrylic acid given was the highest which was not followed by toxic effects resulting in death (or visible illness) during an observation period of 3 weeks. Thus, unlike mice and guinea pigs, swine were
TABLE 1. Protection of guinea pigs by polyacrylic acid against foot-and-mouth disease (FMD) virus lesions

| FMD virus dilution | Time after virus administration (days) | Guinea pig reaction after various amounts of polyacrylic acida | 10 mg | 2.5 mg | None |
|--------------------|----------------------------------------|---------------------------------------------------------------|-------|--------|------|
|                    |                                        |                                                               |       |        |      |
|                    |                                        | Lesionsb | Diedc | Lesionsb | Diedc | Lesionsb | Diedc |
| 10^{-3}            |                                        | 0 | 0 | 0 | 0 | 4 | 0 |
| 10^{-2}            |                                        | 0 | 0 | 0 | 0 | 2 | 0 |
| 10^{-1}            |                                        | 0 | 0 | 0 | 0 | 2 | 0 |

a A 0.1-ml amount of virus dilution administered in the foot pad 18 hr after intraperitoneal administration of polyacrylic acid.
b Per group of four, number of animals showing generalized FMD lesions.
c Per group of four, number of animals which died due to toxicity of polyacrylic acid. Toxicity was similar in guinea pigs which did not receive FMD virus.

not protected at all against FMD virus by polyacrylic acid administered intraperitoneally.

FMD Virus Infection and COAM. COAM and a number of related compounds derived from natural polysaccharides are markedly less toxic than polyacrylic acid (2, 4). This and other properties make these newly described compounds more interesting for use as antiviral agents. COAM was found to protect mice against FMD virus when used under conditions as described for polyacrylic acid. The activity of the compound against a small virus dose was studied in detail. Three litters of eight mice were given COAM intraperitoneally, 10 to 300 µg, or 2 mg per kg to 60 mg per kg per suckling mouse of 5 g. After 18 hr they received one LD_{50} of FMD virus by the same route. Mortality was delayed in all mice which had received COAM. The pooled results are given in Fig. 2. The protection given by the lowest dosage, 2 mg/kg, was significant.

Swine used for protection experiments weighed between 17 and 25 kg, with an average of 20 kg. In initial experiments, it seemed that COAM-treated swine were unusually susceptible to death caused by handling stress. However, deaths also occurred in untreated animals submitted to frequent or energetic experimental handling. The number of deaths in COAM-treated animals became negligible when handling stress was reduced (Table 2). The animals showed no gross ill effects after intraperitoneal or subcutaneous administration of 30 mg/kg and 120 mg/kg of COAM for observation periods of 3 weeks. Higher dosages were not tested. At autopsy, fibrin deposits were found on the peritoneum of a majority of intraperitoneally treated animals. No or minimal local lesions were found after subcutaneous administration of COAM.

COAM-treated and control animals were infected through contact with pigs which had received FMD virus intramuscularly (day 0) and developed a confluent picture of FMD on days 2 and 3. Control animals showed symptoms shortly afterwards (Table 2). Twelve pigs were given 30 mg of COAM per kg intraperitoneally 7 days and 18 hr before exposure. They developed lesions simultaneously with the control animals. With 120 mg of COAM per kg, there was no significant difference between treated and untreated animals with regard to the time that the first foot lesions appeared and proceeded to all extremities (Table 2), the number and extent of lesions, and temperature measurements. At autopsy on day 10, lesions were found on snout, tongue, and heart to the same extent in treated and untreated animals.

The appearance of virus-neutralizing antibodies was examined on day 10. Pre-exposure sera had titers of less than 2. Postinfection sera had titers between 2 and 16, with a geometric mean titer of 3.8 in COAM-treated animals. There was no difference between the different COAM-treated
groups. In untreated animals, postinfection sera had titers of 2 to 8, with a geometric mean of 3.6. Thus no difference in antibody response between COAM-treated and control animals was noted. COAM at a dosage 60 times greater than the effective dose in mice had no measurable effect against FMD in swine.

Search for interferon in COAM-treated swine. Conceivably, the lack of anti-FMD effect of polycarboxylates in swine could be due to failure to induce interferon in these animals. Interferon induction by COAM in swine was therefore investigated. A male and female pig, each weighing 20 kg, received 120 mg of COAM per kg intraperitoneally. Blood samples were taken after 0, 6, 12, 18, 24, and 36 hr. This schedule was adopted since mice have highest titers of circulating interferon 18 hr after intraperitoneal injection of carboxylate polymers (4, 10, 14). Serum was stored at −20°C and assayed for interferon by VSV plaque and yield reduction on primary swine kidney cells and on IB-RS-2 cells. After 18 hr of pretreatment with doubling dilutions of serum starting at 1:8, cells were washed and challenged with VSV. There was no plaque reduction or yield reduction of VSV by the test sera compared with control sera taken at 0 hour or with untreated controls. The VSV-swine cell system was sensitive to interferon for the following reasons. (i) Primary swine kidney cells, treated for 3 hr with 10 μg of poly 1:C (PL-Biochemicals) per ml (12) and 30 μg of diethylaminoethyl (DEAE)-dextran per ml (11), and then washed and further incubated for 18 hr, released a factor in the medium which suppressed plaque formation of VSV in the same types of cells when used at a 1:8 dilution in an interferon assay; this factor had properties of interferon. (ii) A much lower concentration of poly 1:C, 0.1 μg/ml, induced cellular resistance to VSV; DEAE-dextran was needed for the effect. (iii) Recently, primary swine kidney cells and IB-RS-2 cells were found sensitive to swine interferon when FMD virus was used for assay (17). On the other hand, there is no record of VSV being insensitive to interferon in primary cultures of normal cells. It is concluded that swine did not react with detectable serum interferon to COAM.

Hog cholera and COAM. The marked discrepancy between the effects of COAM on FMD virus infection in small rodents and in swine also led us to investigate protective effects in a different viral infection of swine. Hog cholera was chosen as another infectious system. The virus is of major importance in swine, the only animal in which it provokes symptoms. Its properties are entirely different from those of the FMD rhinovirus, and although unclassified it shares many biophysical and biological characteristics with rubella virus (16). Natural exposure was not deemed advisable in the present experiment since this resulted in symptoms arising over a prolonged period in different animals and difficulty of comparison between test and control groups. Swine weighing 20 kg were randomized, and 8 received 120 mg of COAM per kg intraperitoneally. After 18 hr, the COAM-treated and eight control pigs were given intramuscularly 1,000 LD50 units of hog cholera virus, as previously determined in vivo. The maximum rectal temperature was reached on days 3, 4, or 5 in control animals. This temperature was attained in none of the test animals until 6 days after virus administration. The average temperature curves are charted in Fig. 3. Diarrhea was first noticed in control animals on days 2 and 3 and in COAM-treated animals on days 5 and 6. Skin lesions appeared in control animals on days 5 and 6 but not before day 7 in treated animals. On day 8, the first swine died; they were two control animals. There was, however, only a very slight difference in average time of death: 10.1 days in control animals and 10.7 days in treated animals. At autopsy, the skin lesion score was lower in treated animals than in controls. Virus was readily demonstrated in the spleen and tonsils

| Administration of COAM (120 mg/kg) | Day of first signs | Four feet affected by day |
|-----------------------------------|--------------------|--------------------------|
| 7 Days before exposure            |                    |                          |
| Intrapertioneally                 | 4a, 3, 4, 4, 4, 4  | 6, 5, 4, 6, 4, 5         |
| Subcutaneously                    | 4, 4, 4, 6, 6, 8   | 6, 5, 6, 6, 9            |
| 18 Hours before exposure          |                    |                          |
| Intrapertioneally                 | 3, 5, 4, 4, 3, 5   | 6, 5, 4, 4, 4            |
| Subcutaneously                    | 4, +b, 7, 5, 6, 5  | 5, +b, 7, 5, 6, 5        |
| No COAM (controls)               | 3, 5, 4, 5, 5, 4   | 3, 6, 4, 6, 6, 4         |

a For each animal in the group, day of first foot lesion after exposure to infected swine.
b Animal died within 36 hr after COAM injection.
given, stranded protect effect against to potential expected. action virus infection. been the lowest against mortality againstmortality been from treated and untreated groups by direct immunofluorescence in PK-15 cells (13).

DISCUSSION

Polyacrylic acid and COAM protected mice against mortality caused by FMD virus. The lowest dosage of COAM given, 2 mg/kg, was effective. It was similar to the lowest effective dose in vaccinia and mengovirus infections (2). The administration schedule of the compounds and the virus was as previously adopted for other viral infections (2, 4, 6, 14) and may therefore have been less than optimal in the case of FMD virus infection. Since the susceptibility of mice to FMD virus decreased drastically after 10 days of age in our assay system, the duration of the protective action could not be determined.

The activity of interferon-inducing polycarboxylates in FMD virus infection is not unexpected. FMD virus has shown good sensitivity to interferon in cells from all species tested, also including mice (18) and swine (17). The first potential inducer of interferon used to protect against FMD virus was yeast RNA. At 350 mg per kg per day in multiple injections, it delayed symptoms in guinea pigs (3), and a protective effect was also evidenced in mice and cattle (19). Recently, microgram amounts of the double-stranded synthetic RNA, poly I:C, were found to protect mice against FMD virus infection (18).

Surprisingly, swine did not react to COAM given in amounts up to 60 times greater than the effective dose in mice. COAM, 120 mg/kg given 18 hr and 1 week prior to natural exposure to pigs injected with FMD virus, did not delay or attenuate the symptoms of disease. Polyacrylic acid, a plastic polymer less likely than COAM to undergo breakdown in vivo (2), also lacked activity when given under comparable conditions.

A major difference between the experiments in swine and in mice was that swine received the compound intraperitoneally and virus by contact, whereas mice were given the compound and the virus successively in their peritoneal cavity. In the latter situation, interference by residual COAM or polyacrylic acid with viral adsorption or penetration or direct interaction with the virus might have occurred. This phenomenon was demonstrated in vitro with other viruses, and it may account for part of the in vivo antiviral effect (2, 9). The virus dosage effect observed (Fig. 1) is compatible with such an explanation. However, polycarboxylates also protect mice when given by another route than the route of virus administration (2, 14), although less effectively than when the compound and the virus are both given intraperitoneally.

The experiments with guinea pigs prove that animals may be protected by the intraperitoneal route against FMD virus introduced by a parenteral route. Unlike treated swine infected by contact, treated guinea pigs infected by way of the foot pad showed protection against the development of FMD lesions.

The lack of detectable interferon after COAM administration in swine could be related to its failure to modify FMD in this animal. However, polycarboxylates impart antiviral protection in many situations in which circulating interferon is not found after administration. Only the higher doses induce demonstrable interferon, whereas long-term protection is also given by the lower doses. The smallest interferon-inducing dose of polyacrylic acid in mice is 20 mg/kg (10). Mice are protected against vaccinia lesions with 2 mg/kg (6). Among antivirally active chlorite-oxidized oxypolysaccharides, the chemical group to which COAM belongs, only three out of seven induced demonstrable interferon (4). With polyacrylic acid, we were unable to demonstrate interferon production in rabbits and in rats. Vaccinia lesions were inhibited in rabbits, and polyoma virus induced fewer tumors in rats after treatment with the polymer (8). Sellers (personal communication) found an antiviral effect of pyran copolymer in FMD virus infection in guinea pigs, but serum taken after pyran administration did not protect guinea pig cells. The absence of detectable interferon, therefore, has limited value in explaining the lack of anti-FMD effect of polycarboxylates in swine. In addition, there is no ground to postu-
late general unresponsiveness of the interferon system. As has been shown, the interferon system is induced in swine cells by double-stranded RNA, and swine leukocytes produce interferon when exposed to phytohemaggutinin, a weak inducer in other species (17). FMD virus is sensitive to swine interferon in homologous cells (17).

The experiment with hog cholera indicates that COAM exerts protective activity in swine in certain viral infections. In each of the treated animals, temperature rise, diarrhea, and skin lesions were delayed when compared to untreated swine. The effect was limited, and there was no survival of significant duration.

The effect of polycarboxylates on FMD virus infection of mice and guinea pigs and their lack of effect in swine are exemplary of the hazard of extrapolating observations obtained with experimental infection of unnatural hosts to naturally occurring disease. To our knowledge, this is the first example of activity of interferon inducers in the experimental host and lack of activity in a natural host.

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