Effect of Antiviral Agents in Equine Abortion Virus-Infected Hamsters

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Equine abortion virus, a member of the herpesvirus group, produces a lethal infection in hamsters. With this system, the protective effect of certain inhibitors of deoxyribonucleic acid viruses, inducers of interferon and exogenous interferon, was evaluated. Of the various agents studied, 9-β-d-arabinofuranosyladenine markedly suppressed mortality, and 5-iodo-2'-deoxyuridine, distamycin A, and N-ethylisatin β-thiosemicarbazone were inactive. Of the inducers tested, statolon, ultraviolet-irradiated Newcastle disease virus, and polyriboinosinic:polyribocytidylic acid (poly I:C) were protective, and endotoxin, polyacrylic acid, and polymethacrylic acid did not protect. Administration of exogenous interferon did not afford protection. Statolon and ultraviolet-irradiated Newcastle disease virus induced circulating interferon in hamsters, whereas poly I:C, endotoxin, and polyacrylic acid did not produce interferon. Because of the severity of the disease produced in hamsters by equine abortion virus, lack of protective activity by an agent in this system should not preclude possible efficacy against other members of the herpesvirus group.

Equine abortion virus (EAV) or equine rhinopneumonitis virus is a member of the herpesvirus group. Doll and co-workers (5) first adapted EAV to Syrian hamsters, and Randall and Bracken (15) described the growth cycle of the virus in hamsters characterized by a viremia and fulminating hepatitis followed by death.

In the present study, the protective effect of inducers of interferon and selected inhibitors of deoxyribonucleic acid (DNA) viruses was evaluated in hamsters experimentally infected with EAV. Serum interferon levels in hamsters injected with interferon inducers were also determined. Although certain antiviral agents were able to protect EAV-infected hamsters, the fulminating nature of the disease renders it a model of limited usefulness in evaluating the efficacy of antiviral agents.

MATERIALS AND METHODS

Viruses. A single pool of EAV-infected hamster liver was prepared from virus obtained from R. W. Darlington, St. Jude Children's Research Hospital, Memphis, Tenn. A 0.2-ml volume of a 10^-1.0 dilution was inoculated intraperitoneally (ip) into hamsters weighing 30 to 35 g, and livers were collected 22 hr postinfection from moribund animals. The livers were triturated as a 10% suspension in phosphate-buffered saline (PBS) containing 2% bovine serum albumin, sealed in ampoules, and stored at -60 C. For most studies, hamsters under light ether anesthesia were infected by ip injection with 1 to 13 LD50 of virus, and they usually died in 3 to 7 days. Larger doses of virus killed animals as early as 24 to 48 hr after injection.

The Indiana strain of vesicular stomatitis virus (VSV) was obtained from S. Baron, National Institutes of Health, Bethesda, Md. A stock virus was propagated in L-929 cells and stored at -60 C.

Hamsters. Male Syrian hamsters weighing 30 to 35 g were supplied by Lakeview Hamstery, Newfield, N.J. Infected hamsters were usually housed in individual cages to prevent reinfection and death due to cannibalism of infected, dead animals. Deaths were recorded over an observation period of 14 days.

Compounds. Statolon, an inducer of interferon, was obtained through the generosity of W. J. Klein-schmidt, Lilly Research Laboratories, Indianapolis, Ind. The material was dissolved in 1% NaHCO3 solution. A 250 mg/kg dose contained 23.75 mg of active statolon per kg in 0.5 ml. Other inducers of interferon used included polyriboinosinic:polyribocytidylic acid (poly I:C), lot no. 11-8-321, purchased from Miles Laboratories, Elkhart, Ind., and solubilized in physiological saline. Poly I:C, lot no. 2026, purchased from Pabst Laboratories, Milwaukee, Wisc., was reconstituted in sterile distilled water. Polyacrylic acid was prepared by P. Daniels, Schering Corp., Bloomfield, N.J., and polymethacrylic acid was obtained from Paul Valley Industrial Park, Warring-
ton, Pa. The endotoxin used was lipopolysaccharide \textit{W. coli:026:B6} purchased from Difco Laboratories, Detroit, Mich. Egg-propagated Newcastle disease virus (NDV) with a titer of 10^9 plaque-forming units per ml was irradiated with ultraviolet light to a survival of <.001%. The interferon inducers were administered prophyactically either 1 day or 2 or 3 hr before virus infection, generally as a single dose.

Inhibitors of DNA-containing viruses were 9-beta-arabinofuranosyladenine (ara-A), obtained from Pfanstiehl Laboratories, Waukegan, Ill.; distamycin-A, kindly supplied by M. Ghione of Farmitalia, Milan, Italy; 5-iodo-2'-deoxyuridine (IUdR) from International Chemical and Nuclear Corp., Los Angeles, Calif.; and N-ethylisatin 6-thiosemicarbazone (NEITC) from Schering Corp. Ara-A was injected ip 1 hr before infection, 4 hr postinfection, and twice daily for 4 days. Distamycin A was administered subcutaneously (sc) 1 hr before and 1 hr after infection and once daily for 2 additional days. IUdR was given sc 1 hr preinfection, 1 hr postinfection, and twice daily on the succeeding day. NEITC was injected sc as a single dose 2 hr after infection. The dose regimen and routes of treatment were selected because they had previously been reported protective in a number of other in vivo systems (6, 14, 20, 23).

Preparation of exogenous interferon. Hamster brain interferon was prepared by inoculation of West Nile virus in hamsters as described by Frickey (7). Brains from paralyzed hamsters were collected and homogenized as a 20% suspension in Hanks balanced salt solution, centrifuged at 400 × g to remove debris, and dialyzed at 4°C (pH 2.0) for 24 to 72 hr and then against PBS (pH 7.2) for 24 hr. Brains injected with PBS were processed similarly and served as control. Both preparations were centrifuged after dialysis to remove precipitate. Exogenous interferon had a titer of 400 to 800 units/ml when assayed, as described below, on primary hamster kidney cells.

Hamsters were prophylactically treated with 1- or 2-ml doses of interferon ip 2 hr preinfection, and a second dose was administered 2 hr postinfection. In one test, a third dose was given on the day after infection.

Cell cultures and medium. Primary hamster kidney cell cultures prepared from 31- to 57-day-old Syrian hamsters were obtained from Grand Island Biological Co., Grand Island, N.Y., as a suspension of 10^6 cells/ml in Hanks lactalbumin hydrolysate plus 10% fetal calf serum. Petri dishes (10 by 35 mm, Linbro multi-dishes) were seeded with 2 ml of cell suspension in medium supplemented with 100 µg of gentamicin sulfate (Schering Corp.) per ml. Petri dishes were incubated for 3 days at 37°C in 5% CO_2 humidified air, re-fed, and incubated an additional 2 to 3 days. Continuous cell cultures, including two lines of hamster kidney cells, were used: BHK-21-clone 13, and HKCC. The diploid cell line of Chinese hamster lung, DON, was also employed in interferon tests.

Interferon assay. Hamster sera were collected from groups of 10 animals at intervals after injection of interferon inducers and assayed by the plaque inhibition test employing VSV (22). The reciprocal of the highest dilution of 1.0 ml of serum inhibiting 50% of the plaques was considered the titer.

**RESULTS**

Effect of statolon and inhibitors of DNA viruses. The results of a preliminary test designed to determine the protective effect of statolon and certain inhibitors of DNA viruses in the EAV-hamster system are presented in Table 1. A single ip dose of 500 mg of statolon per kg protected 100% of the infected animals housed in groups of 10. Ara-A at dosages of 125 to 250 mg/kg markedly suppressed mortality. IUdR, distamycin-A, and NEITC did not protect at the dosages employed nor was a delay of death observed.

**Effect of inducers of interferon and exogenous interferon**. The protection seen with statolon prompted evaluation of the efficacy of other inducers of interferon and exogenous interferon. Ara-A was employed as a positive control substance. Hamsters were housed singly in individual cages. The results of two experiments are shown in Table 2. Statolon demonstrated a protective effect over the dose range of 62.5 to 250 mg/kg. In hamsters treated ip 3 hr preinfection with a single dose of poly I:C from Miles Laboratories, protection was observed in one test, but, in a second test employing poly I:C from Pabst Laboratories, no protection was observed. The reason for this difference was not studied further, but it is recognized that poly I:C preparations can be heterogeneous. A single intravenous (iv) injection of ultraviolet (UV)-irradiated NDV administered 1...
Table 2. Activity of inducers of interferon, exogenous interferon, and ara-A in equine abortion virus*-infected hamsters

<table>
<thead>
<tr>
<th>Drug*</th>
<th>Dose</th>
<th>No. of doses</th>
<th>Expt 1</th>
<th>Expt 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>S/T*</td>
<td>% Survivors</td>
</tr>
<tr>
<td>PBS</td>
<td>1.0 ml</td>
<td>2</td>
<td>1/10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>62.5 mg/kg</td>
<td>1</td>
<td>9/10</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>125.0</td>
<td>1</td>
<td>9/10</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>250.0</td>
<td>1</td>
<td>7/10</td>
<td>70*</td>
</tr>
<tr>
<td>Poly I:C</td>
<td>10.0 μg</td>
<td>1</td>
<td>9/10</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
<td>1</td>
<td>9/10</td>
<td>90</td>
</tr>
<tr>
<td>UV-NDV</td>
<td>0.2 ml</td>
<td>1</td>
<td>2/10</td>
<td>20</td>
</tr>
<tr>
<td>Exogenous IF</td>
<td>1.0 ml</td>
<td>3</td>
<td>2/10</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>0.1 ml</td>
<td>2</td>
<td>2/10</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>0.2 ml</td>
<td>2</td>
<td>2/10</td>
<td>20</td>
</tr>
<tr>
<td>Normal Brain</td>
<td>1.0 ml</td>
<td>3</td>
<td>1/9</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2.0 ml</td>
<td>2</td>
<td>1/9</td>
<td>11</td>
</tr>
<tr>
<td>Ara-A</td>
<td>125.0 mg/kg</td>
<td>8</td>
<td>9/10</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>250.0</td>
<td>10/10</td>
<td>10/10</td>
<td>100</td>
</tr>
</tbody>
</table>

\* 1.6 to 2.0 LD₅₀ of virus.
\* Abbreviations: PBS, phosphate-buffered saline; Poly I:C, polyriboinosinic:polyribocytidylic acid; UV-NDV, ultraviolet-irradiated Newcastle disease virus; IF, interferon; ara-A, 9-β-D-arabinofuranosyladenine.
\* Number of survivors/total number infected.
\* Poly I:C (Miles Laboratories).
\* Poly I:C (Pabst Laboratories).

Day prior to infection was protective. Exogenous interferon was inactive in both trials, whereas ara-A demonstrated a marked protective activity.

In several experiments not shown here, polyacrylic acid was inactive when three doses of 60 mg/kg were given prophylactically by the sc route or with a single ip injection of 120 mg/kg administered 1 day preinfection. Polymethacrylic acid was without effect when a single dose of 120 mpk was given 1 day prior to infection. A single ip dose of 100 μg of endotoxin administered 2 hr preinfection was also inactive. Although statolon was protective when injected ip 1 day preinfection, it was ineffective when treatment was given 3 hr before infection. Subcutaneous administration 1 day preinfection reduced efficacy.

Induction of interferon in hamsters. Of the various interferon inducers, statolon, Poly I:C, and endotoxin were injected ip, UV-irradiated NDV was administered iv, and polyacrylic acid was given sc to hamsters. At various intervals, sera were collected and assayed for interferon content on primary hamster kidney and continuous cell lines of BHK-21-clone 13, HKCC, and DON. The results on primary hamster kidney cells are presented in Table 3. Sera from statolon and UV-irradiated NDV-injected hamsters had interferon titers of 160 units/ml or more when assayed on primary hamster kidney cultures but were negative on BHK-21-clone 13, HKCC, and DON cells. Poly I:C, polyacrylic acid, and endotoxin did not produce detectable amounts of interferon in hamster sera, although the same lots of inducers.

Table 3. Hamster serum interferon titer following of various inducers

<table>
<thead>
<tr>
<th>Inducers*</th>
<th>Dose</th>
<th>Route*</th>
<th>Time (hr) after dose</th>
<th>Interferon (units/ml)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statolon...</td>
<td>250 mg/kg</td>
<td>ip</td>
<td>16</td>
<td>160</td>
</tr>
<tr>
<td>UV-NDV...</td>
<td>0.2 ml</td>
<td>iv</td>
<td>8</td>
<td>&gt;160</td>
</tr>
<tr>
<td>Poly I:C (Miles)...</td>
<td>100 μg</td>
<td>ip</td>
<td>6</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Poly I:C (Pabst)...</td>
<td>100 μg</td>
<td>ip</td>
<td>6</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Escherichia coli endotoxin...</td>
<td>100 μg</td>
<td>ip</td>
<td>2</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Polyacrylic acid...</td>
<td>60 mg/kg</td>
<td>sc</td>
<td>24</td>
<td>&lt;10</td>
</tr>
<tr>
<td>PBS...</td>
<td>0.2 ml</td>
<td>ip</td>
<td>6</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

\* Assayed on primary hamster kidney cells.
\* For abbreviation, see footnote b, Table 2.
\* ip, Intraperitoneal; iv, intravenous; sc, subcutaneous.
stimulated circulating interferon in mice. The titers in mice ranged from 160 international units (IU)/ml for polyacrylic acid to 400 IU/ml for poly I:C (Pabst) and 1,500 IU/ml (Miles).

DISCUSSION

In this study, the protective action of various interferon inducers and several inhibitors of DNA-containing viruses was evaluated in hamsters infected with EAV, a member of the herpesvirus group.

In agreement with earlier reports of the antiviral activity of ara-A against herpes simplex virus in hamsters (17–19), mice (17, 20), and rabbits and monkeys (11), ara-A demonstrated a striking antiviral effect in the EAV-hamster model. IUDR (10) and distamycin A (23), which inhibit herpes simplex virus infections in rabbits, failed to protect in the EAV-hamster system. NEITC, known to be active against pox viruses and inactive against herpes virus (2), was ineffective against EAV.

Statolon demonstrated potent protective activity, UV-irradiated NDV and poly I:C showed inhibitory action in certain trials, but polyacrylic acid, polymethacrylic acid, and endotoxin were inactive. The mechanism responsible for the varied spectrum of protective action by these interferon inducers was not the subject of this report. Other investigators (8, 13), however, have reported that certain herpesviruses are relatively resistant to the antiviral action of interferon or interferon inducers. The variability of protection might better be explained by qualitative or quantitative differences of the inducers. It is now recognized that, as reviewed elsewhere (3), inducers of interferon stimulate a multiplicity of host-defense responses in addition to the induction of circulating interferon.

Statolon and UV-irradiated NDV induced circulating interferon in hamsters, but poly I:C, polyacrylic acid, and endotoxin failed to produce detectable interferon. The findings with UV-irradiated NDV and poly I:C are in agreement with previous reports (1, 12, 16). The failure of poly I:C to stimulate interferon production in hamsters is in disagreement with the results of Stewart et al. (21). However, the present findings may simply reflect insensitivity of the assay system employed or the inability of the hamsters to respond to certain interferon inducers. Moreover, it was recently reported that a single autosomal factor was responsible for differences in interferon production of various strains of mice (4). Similarly, it might be expected that a different strain of hamster might respond differently to interferon inducers.

It is possible that the administration of more potent preparations of exogenous interferon repeated continuously during the course of infection might have achieved increased protection, as was observed by Gresser et al. (9) in mice infected with encephalomyocarditis virus.

The relevance of this model to the evaluation of antiviral drugs against herpes-type viruses may be limited, although ara-A, which protects against Herpesvirus hominis, is protective in this system. EAV produces an extensive, severe disease in hamsters, and the lack of protective activity by an agent against this herpesvirus does not preclude efficacy in experimental models employing other members of the herpesvirus group.

LITERATURE CITED

17. Schabel, F. M., Jr. 1968. The antiviral activity of 9-B-D-


