Effect of Poly I:C on Infectious Bovine Rhinotracheitis Virus Infection in Calves[1] (35750)

K. W. Theil, S. B. Mohanty, and F. M. Hetrick

Departments of Veterinary Science and Microbiology, University of Maryland, College Park, Maryland 20742

The synthetic double-stranded RNA, polyinosinic·polycytidylic acid (poly I:C) induces resistance to vesicular stomatitis virus (VSV) in bovine kidney cell cultures (1) and host resistance to foot and mouth disease virus in mice (2). Poly I:C has also been shown to inhibit human respiratory viruses *in vitro* (3) and respiratory viruses of animals *in vivo* (4). An important question is: Will poly I:C treatment provide calves with protection against viruses that cause respiratory disease? Infectious bovine rhinotracheitis (IBR) virus causes a respiratory disease in cattle characterized by an intense inflammation of the upper respiratory tract accompanied by dyspnea, depression, nasal discharge, and loss of condition (5). The present experiments were undertaken to assess the effect of pretreating calves with poly I:C on subsequent challenge with IBR virus.

Materials and Methods. Test Animals. A total of 7 bull calves, 6–8 weeks of age, were used in 2 experiments. All calves were seronegative for IBR virus and negative for virus isolation at the start of the experiment.

Virus. The N-1 strain of IBR virus propagated in bovine embryonic kidney (BEK) cells was used as the challenge virus.

Poly I:C. Poly I:C obtained from Biopolymers Laboratory, Chagrin Falls, Ohio, was used in Expt. 1. This polymer had a hypochromicity of 37% at 248 mμ. As large amounts of poly I:C were required for Expt. 2, poly I:C was obtained from a more economical source (P. L. Biochemicals, Inc., Milwaukee, Wisconsin). This preparation had a hypochromicity of 38% at 248 mμ and absorbance ratio (280 mμ/260 mμ) of 0.62 at pH 7.0. In both experiments, poly I:C was administered intravenously (iv) to the calves.

Expt. 1. This was a preliminary experiment to assess qualitatively the effect of poly I:C on IBR virus infection of calves. One calf was given poly I:C (0.5 mg/kg of body wt) iv, 3 hr prior to intranasal (in) instillation of IBR virus while the other calf did not receive poly I:C prior to infection. Intranasal instillation of virus inoculum was performed by the method previously described (6). After inoculation, calves were examined daily for signs of respiratory illness and rectal temperatures were taken twice daily and averaged.

Expt. 2. Five calves were allotted to 3 groups and housed in separate isolation stalls. Calves 1 and 2 were controls and received no poly I:C. Three hr before infection, poly I:C was administered iv to calves 3 and 4 (0.5 mg/kg body wt) and calf 5 (1.0 mg/kg body wt). Each calf was infected by in instillation of $10^{7.8}$ $TCID_{50}$ of IBR virus.

Serum for putative interferon assay was collected at hourly intervals after poly I:C administration until calves were infected and at 24 hr postinfection. Serum from untreated calves was taken prior to inoculation and at 24 hr postinfection. Sera were assayed for putative interferon by the 50% plaque reduction method using VSV and a BEK cell line. Briefly, monolayers were treated overnight with 2 ml of serial 2-fold dilutions of sera made in minimum essential medium and then washed with Hanks' balanced salt solution (HBSS) prior to adsorption of VSV. After adsorption, overlay medium was added and plaques were counted at 40 hr. Putative in-

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terferon titers were expressed as reciprocals of the dilutions which reduced the number of virus plaques 50%.

Nasal swabs were taken on day 1 and every day continuing through day 5 and on days 7 and 9. Following collection, all specimens were immersed in 1.5 ml of HBSS containing antibiotics and stored at —70° until titrated in primary BEK cell culture. Temperatures were taken and daily observations were made as described in Expt. 1.

On day 15 postinoculation, one calf from each group was euthanatized and necropsied. Tissues were taken from the lung and preserved for histopathologic examination.

Results and Discussion. Expt. 1. This preliminary experiment indicated that poly I:C did provide a significant degree of protection against IBR virus infection. The untreated calf developed severe respiratory illness characterized by depression, copious nasal discharge, coughing, rales in both lungs, and a marked febrile response (highest av daily temp, 41.6°). The poly I:C-treated calf had much milder clinical manifestations of respiratory illness with little nasal discharge, moderate febrile response (highest av daily temp, 41.0°), and no rales or depression.

Expt. 2. Treatment of calves with poly I:C did not prevent IBR virus infection but did alter the severity of respiratory disease (Table I). Throughout the experiment, the overall condition of the treated calves was far superior in comparison to the untreated calves. The untreated calves developed severe respiratory illness while the treated calves had much milder clinical symptoms. Calves 3 and 4 had reduced febrile responses, but calf 5 had a febrile response similar to that of the untreated calves, though its onset was delayed by 1 day. The amount of virus shed in the nasal secretions on day 1 postinfection was markedly less in treated calves, but by day 2 all calves, except calf 4, were shedding comparable amounts of virus. From days 3 through 9, the amount of virus shed was approximately the same for all calves.

Poly I:C treatment either reduced or prevented pneumonic lesions in the lungs, depending on the dosage used. The larger dose provided complete protection. Poly I:C induced only modest levels of putative circulating interferon. Putative circulating interferon was not detected in the untreated calves at the time of infection nor in treated calves 1 hr afer poly I:C was administered. Calves 3, 4, and 5 had putative interferon titers 8, 4, and $\geqq 8$, respectively, at the time of infection. Calf 4 had a titer of 8 at 2 hr after poly I:C treatment, but the titer dropped to 4 by the time of infection. Putative interferon was not detected in the sera of any of the calves at 24 hr postinfection.

The protective effect of poly I:C against animal virus infections *in vivo* and *in vitro* is well documented (1–4). The present studies extend these observations by demonstrating the partial protection of a large domestic animal by poly I:C against respiratory virus disease. The inability of large doses of poly I:C to afford complete protection against IBR virus infection is possibly attributable to the poor response of calves to interferon induction by this compound or maybe a reflection of the severity of the viral challenge. Rosenquist (7) has also reported relatively low serum interferon responses in calves injected iv with poly I:C; however, the protective effect of poly I:C against virus challenge was not determined in this study.

The reason for this poor response is not known. However, calf kidney cell cultures require considerably larger amounts of poly I:C to induce resistance to VSV than cell cultures derived from certain other species (1). The low levels of interferon induced by poly I:C in calves may be a reflection of this intrinsic refractoriness of bovine cells to poly I:C induced resistance.

Summary. Poly I:C administered iv 3 hr prior to challenge with IBR virus did not prevent infection but did alter the severity of respiratory disease. Poly I:C-treated calves had much milder clinical manifestations of respiratory illness than the untreated controls. Depending on the dosage used, Poly I:C reduced or prevented the pneumonic lesions in the lungs. Complete protection from these lesions was provided by a dose of 1 mg/kg of body wt. Poly I:C induced only modest levels of putative circulating interferon in calves.

TABLE I. Effect of Poly I:C on IBR Virus Infection of Calves.

Calf	Dosage of poly I:C (mg/kg of body wt)	Maximum putative interferon titer[a] [b]	Virus titer from nasal swabs (log_{10} $TCID_{50}$/0.1 ml)							Clinical response[c]	Necropsy and Histopathologic changes in lungs
			1	2	3	4	5	7	9		
1	None	<4	5.0	5.5	4.5	5.0	4.5	3.5	1.0	Severe; temp, 41.3°	Not done
2	None	<4	5.0	5.5	5.0	5.5	5.5	1.5	<1.0	Severe; temp, 41.6°	Large areas of consolidation and pneumonia
3	0.5	8(3)	<1.0	5.5	5.5	5.5	4.5	3.5	<1.0	Mild; temp, 40.8°	Not done
4	0.5	8(2)	<1.0	3.5	4.0	6.3	3.0	3.0	1.5	Mild; temp, 40.7°	Small areas of consolidation and pneumonia
5	1.0	≧8(3)	2.0	5.5	5.5	4.5	4.5	4.0	2.0	Very mild; temp, 41.4°	No change

[a] Units/2 ml.

[b] Numbers in parentheses represent the hour after poly I:C administration at which maximum titer was detected.

[c] Temperatures represent the highest average daily temperature recorded for each calf.

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