Observations of Cattle, Goats and Pigs after Administration of Synthetic Interferon Inducers and Subsequent Exposure to Foot and Mouth Disease Virus

J. W. McVicar, J. Y. Richmond, C. H. Campbell and L. D. Hamilton*

ABSTRACT

Polyriboinosinic-polyribocytidylic acid (poly [rI.rC]) was administered intravenously to 11 cattle and 13 goats in doses of 0.25 to 4.0, and 1.0 to 5.0 mg/kg, respectively. Subsequent exposure of these and untreated control animals to foot and mouth disease virus (FMDV) failed to demonstrate any differences in either the course or severity of the disease. Serum interferon was detected in cattle one hour after the intravenous administration of poly (rI.rC).

Six pigs given 4, 20, or 100 mg/kg of itaconic-acrylic acid copolymer (IAA, HMW) intraperitoneally reacted clinically the same as six untreated control pigs after contact exposure to FMDV.

Three pigs given 50, 100, or 200 mg/kg of divinyl ether-maleic anhydride copolymer (DVE/MA, pyran) intraperitoneally similarly failed to show any difference in clinical reaction from three untreated control pigs after intranasal instillation of FMDV. Three pigs given 100, 200, or 400 mg/kg of DVE/MA intraperitoneally developed rapid diffuse peritonitis causing the death of one in 48 hours.

RÉSUMÉ

On a administré de l'acide polyriboinosinique-polyribocytidylique (poly [rI.rC.]), par la voie intra-veineuse, à 11 bouvillons et à 13 chèvres, aux doses respectives de 0.25 à 4.0 et de 1.0 à 5.0 mg/kg. L'inoculation subséquente du virus de la fièvre aphteuse à ces bêtes et à des animaux témoins ne produisit aucune différence dans le cours ou la gravité de la maladie. On décela de l'interféron dans le sérum des bouvillons, une heure après l'injection d'acide poly (rI.rC).

Six porcs auxquels on avait injecté, par la voie intra-péritonéale, 4.0, 20 ou 100 mg/kg du copolymère de l'acide itaconique-acrylique réagirent cliniquement à une infection par contact avec le virus de la fièvre aphteuse, de la même façon que six porcs témoins.

Trois porcs ayant reçu, par la voie intra-péritonéale, 50, 100 ou 200 mg/kg du copolymère anhydre du divinyl éther-maléique (DVE/MA, pyran) ne manifestèrent également aucune différence dans leur réaction clinique, comparativement à trois porcs témoins, à la suite d'une instillation intranasale du virus de la fièvre aphteuse.

Trois autres porcs ayant reçu, par la voie intra-péritonéale 100, 200 on 400 mg/kg de DVE/MA développèrent rapidement une péritonite généralisée qui entraîna la mort de l'un d'entre eux en 48 heures.

*Agricultural Research Service, USDA, Plum Island Animal Disease Laboratory, P.O. Box 848, Greenport, New York 11944 (McVicar, Richmond and Campbell), and Division of Microbiology, Medical Research Center, Brookhaven National Laboratory, Upton, New York 11973 (Hamilton).

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INTRODUCTION

There have been several reports on the effectiveness of certain synthetic compounds in stimulating the *in vivo* production of interferon with concomitant resistance to a variety of infectious agents (5-7, 11, 12, 14, 15, 19). Most of this work has utilized model systems, usually with mice as the experimental animal, whereas reports of experiments with domestic animals have been few. In 1963, Thely *et al* (30) observed that the administration of ribonucleic acid (RNA) from yeast prolonged survival in mice and delayed appearance of lesions in guinea pigs and cattle infected with foot and mouth disease virus (FMDV). These effects may have resulted from interferon induced by the yeast RNA. Interferon has been detected in the serum of calves after the injection of a number of viral and nonviral inducers including polyriboinosinic-polyribocytidylic acid (poly [rI.rC]) (24-26). Interferon has also been detected in the nasal mucus of calves after intranasal administration of poly (rI.rC) (2).

Two reports describe experiments in which animals were treated with synthetic interferon inducers and then exposed to virus. Leunan *et al* (12) showed that injection of polyacrylic acid or chlorite-oxidized oxyamylose (COAM) failed to protect pigs against contact-acquired foot and mouth disease (FMD) although both compounds protected mice against FMDV infection. COAM, however, delayed the onset of hog cholera in a group of pigs. Sellers *et al* (27) reported that pyran administered intraperitoneally (IP) gave cattle or pigs little or no protection against contact infection with FMD, whereas guinea pigs were protected to some degree.

Experiments in this laboratory showed that injections of poly (rI.rC) (22), itaconic-acrylic acid copolymer (IAA), referred to in the literature (4, 19) as HMW, or divinyl ether-maleic anhydride copolymer (DVE/MA), referred to in the literature as pyran, protected suckling mice from the lethal effects of FMDV infection (23). We report here on experiments designed to show if injections of these compounds would protect cattle, goats, or pigs subsequently exposed to FMDV. Clinical observations and results of pathological examinations after administration of the compounds and after exposure to FMDV will be given.

MATERIALS AND METHODS

Experimental Animals

Grade Hereford steers, one to two years old and weighing from 200 to 400 kg, were used. Goats were young adults of mixed breed weighing from 20 to 40 kg. Tamworth pigs were used which varied in weight in different experiments. Those given injections of IAA averaged 18 to 20 kg; one group given DVE/MA weighed 65 to 90 kg and the other 110 to 130 kg.

Virus Challenge

FMDV, type O, subtype 1 was used in all experiments. Animals were exposed as follows: cattle, intradermalingual (IDL) or intramuscular (IM) injection; goats, intranasal (IN) instillation; pigs IN instillation or contact with infected pigs. In cattle exposed to virus by the IDL route, five replicates of each of four serial tenfold dilutions of the virus were injected into the tongue epithelium. After 24 hours, formation of vesicles at the injection sites was noted so as to provide a titration according to the method of Henderson (10). Total virus dose approximated 10^8 plaque-forming units.

Experimental Compounds

For cattle experiments, poly (rI.rC) complexes, prepared as previously described (22), were dissolved in 0.03 M NaCl. Poly (rI.rC) administered to goats was dissolved in 5% glucose in water (21). Solutions of DVE/MA (lot number XA-124-177, molecular weight 27,000) were prepared in phosphate-buffered saline (PBS) containing 0.001 M Mg++ and Ca++ (23). The IAA (HMW 1246-37) (19) was supplied as an aqueous solution containing 7.61% solids. This carboxylic acid polymer was neutralized with 6 N NaOH and diluted in sterile water before use.

Observations and Sampling

Experimental animals were closely observed after administration of the test compound and after exposure to virus. Serum samples for interferon assay were obtained before and after administration of

the test compound and after virus exposure. Heparinized blood samples for virus assay were obtained daily after virus exposure. Animals which died during the course of the experiments were necropsied immediately. Surviving test animals were necropsied at the end of each experiment. Gross lesions were noted and specimens taken for histopathological examination.

Viremia Studies

Heparinized blood samples were frozen, thawed, and inoculated on previously drained cultures of primary bovine kidney cells. After one hour at 37°C, all cultures were overlayered with Hanks' balanced salt solution containing 0.5% lactalbumin hydrolysate. Samples not producing a cytopathic effect (CPE) after 72 hours at 37°C were considered negative. Positive samples were plaque assayed on bovine kidney cell cultures under agar (3).

Serum Interferon Assay

Preliminary interferon determinations were made by a CPE-inhibition test with 100 $TCID_{50}$ of FMDV as test virus (22). In subsequent assays, vesicular stomatitis virus (Indiana) was used in either a CPE-inhibition (100 $TCID_{50}$) or a plaque-reduction assay (40-80 PFU/bottle). Primary or secondary bovine kidney cells and a swine kidney cell line (IB-RS-2) served as representative homologous cell types.

EXPERIMENTS AND RESULTS

Poly (rI.rC) In Cattle

Cattle experiments with poly (rI.rC) are summarized under A in Table I.

TABLE I. Animals Used in the Experimental Study of Three Interferon Inducers: Summary of Treatment Received and Foot and Mouth Disease Virus Exposure

	Treatment				Virus Exposure		
Experiment	Compound[a]	Route[b]	Animals Treated	Dose	Animals Exposed	Route[b]	Hours After Treatment
A. Cattle	Poly (rI. rC)	IV	2	0.25 mg/kg	2	IDL	2
		IV	4	0.40-0.8 mg/kg	4	IDL	2
		IV	1	0.6 mg/kg	1	IM	2
		IV	1	1.0 mg/kg	1	IDL	6
		IV	1	1.25 mg/kg	1	IDL	2
		IV	1	2.0 mg/kg	1	IDL	6
		IV	1	4.0 mg/kg	1	IDL	2
	PBS	IV	5	—	4	IDL	2
					1	IM	2
B. Goats	Poly (rI. rC)	IV	4	1.0 mg/kg	4	IN	24
		IV	9	5.0 mg/kg	8	IN	24
	5% Glucose	IV	7	—	7	IN	24
C. Pigs	IAA	IP	2	4 mg/kg	2	Contact	<24
		IP	2	20 mg/kg	2	Contact	<24
		IP	2	100 mg/kg	2	Contact	<24
	PBS	IP	6	—	6	Contact	<24
D. Pigs							
Group 1	DVE/MA	IP	1	50 mg/kg	1	IN	48
		IP	1	100 mg/kg	1	IN	48
		IP	1	200 mg/kg	1	IN	48
	PBS	IP	3	—	3	IN	48
Group 2	DVE/MA	IP	1	100 mg/kg			
		IP	1	200 mg/kg			
		IP	1	400 mg/kg			
	PBS	IP	3	—			

[a]Abbreviations: Poly (rI. rC), polyriboinosinic-polyribocytidylic acid; PBS, phosphate-buffered saline; IAA, itaconic-acrylic acid copolymer; DVE/MA, divinyl ether-maleic anhydride copolymer
[b]Abbreviations: IV, intravenous; IP, intraperitoneal; IDL, intradermalingual; IM, intramuscular; IN, intranasal

TABLE II. Serum Interferon Titers in Steers Injected Intravenously with Poly (rI. rC)

Hours after poly (rI. rC) injection	Control	Poly (rI. rC) dose	
		1 mg/kg	2 mg/kg
0	<8[a]	<8	<8
1	<8	16	64
2	<8	64	256
3	<8	64	1024
4	<8	256	>1024
5	<8	256	>1024
6	<8	128	>1024

[a]Reciprocal of highest serum dilution inhibiting CPE of 100 $TCID_{50}$ vesicular stomatitis virus

Observations After Administration of Poly (rI.rC)

Two steers given 1.0 and 2.0 mg/kg of poly (rI.rC) intravenously (IV) had serum samples and rectal temperatures taken hourly for six hours after treatment. Interferon was detected in the serums of both animals at one hour and persisted through six hours (Table II). Temperatures rose 1 to 1.5°F starting one to two hours after the injection and returned to normal five to six hours later. Nine additional cattle received poly (rI.rC) IV. No untoward effects were noted aside from a slight transient fever.

Observations After FMDV Exposure

A steer which had been given 0.6 mg/kg of poly (rI.rC) was inoculated IM with 10^8 plaque-forming units of virus two hours later. An untreated control steer was similarly inoculated. No difference was observed in the clinical reaction of the two steers. Onset and magnitude of viremia and fever and time of appearance and severity of lesions were essentially identical. The remaining ten steers were given virus, either two or six hours after treatment, in the form of an IDL titration. Four control steers received identical inoculations. In no instance was there a discernible difference in clinical reaction between treated and untreated steers. Furthermore, virus titers, calculated from the tongue titrations were not significantly different between the two groups.

Poly (rI.rC) In Goats

Experiments on goats treated with poly (rI.rC) before exposure to FMDV are summarized under B in Table I.

Observations After Administration of Poly (rI.rC)

Thirteen goats received poly (rI.rC) intravenously in doses of either 1.0 or 5.0 mg/kg. The compound, in volumes of 7.5 to 32 ml, was given slowly through a 20-gauge needle. Control animals were given injections of similar volumes of 5% glucose.

Most goats given the higher dose of poly (rI.rC) and some given the lower dose had some reaction. They appeared "anxious" after treatment; several bleated and two lay down suddenly. Other signs frequently observed were urination, usually immediately upon release after the injection; rapid shallow breathing which lasted from 15 minutes to two hours; and an average temperature elevation of 2.3°F in four hours as compared to 0.5°F for control animals. Most treated goats became lethargic, a condition which lasted up to 24 hours in some individuals. Three out of the 13 treated goats had a foul-smelling diarrhea for one to two days. One goat aborted a ⅔ term fetus, two days after treatment. This abortion was one day after virus inoculation and cannot, therefore, be attributed exclusively to the poly (rI.rC).

One goat was found dead 24 hours after being given 5 mg/kg of poly (rI.rC) IV. Necropsy revealed disseminated petechial hemorrhages and free blood in the lumen of the small intestine. The carcass was very thin and the bowel distended with watery ingesta. Examination of tissues from this goat revealed degenerative lesions of the kidney and liver and extensively denuded villi in the jejunum.

Observations After FMDV Exposure

The 12 surviving goats, plus seven sham-treated control animals, were given 10^4 PFU of FMDV intranasally, 24 hours after receiving poly (rI.rC). Treated animals became ill at the same time as untreated ones and developed a clinical picture in all respects identical with that of the control animals. Onset, magnitude, and duration

of viremia were the same in treated and control animals. Pharyngeal samples taken 14 days after virus exposure (17) revealed the same proportion of virus carrier animals in each group. All goats were necropsied three weeks after virus exposure. Examination of tissues from selected animals revealed fatty degeneration of the liver and villi in the jejunum denuded of epithelium.

IAA In Pigs

Six pigs were given 4 to 100 mg/kg of IAA intraperitoneally (Table I,C). Six control animals were given PBS.

Observations After Administration of IAA

No unusual reactions were observed in any of the treated pigs and no gross lesions attributable to the compound were seen at necropsy three weeks after treatment.

Observations After FMDV Exposure

The pigs were exposed to FMDV as follows: four hours after treatment, an untreated pig was placed in the same isolation unit and inoculated with $10^{4.6}$ mouse ID_{50} of virus in the coronary band of one foot. The virus donor pig was viremic on days 1, 2, and 3 after injection and developed lesions on the uninoculated feet on day 2. Differences in the clinical reactions between treated and untreated pigs exposed to this animal were not observed. Onset of viremia and time of appearance and severity of lesions were the same.

Serum obtained from the donor pig 24 and 48 hours after inoculation had traces of interferon activity. Traces of activity were also detected in the serums of one of the treated pigs 24 and 48 hours after exposure and in another treated pig and two untreated pigs 48 hours after exposure.

DVE/MA In Pigs

Experiments with DVE/MA in pigs are summarized under D in Table I. The pigs of Group 1 weighed 65 to 90 kg and were given 50, 100, or 200 mg/kg of DVE/MA intraperitoneally. The pigs in Group 2, weighed 110 to 130 kg and were given 100, 200, or 400 mg/kg. These latter doses required the administration of relatively large volumes (up to 300 ml) of material. Control pigs received comparable volumes of PBS.

Observations After the Administration of DVE/MA

The Group 1 pigs tolerated the injection of DVE/MA with no apparent discomfort and appeared normal for the next two days. Interferon activity was not detected in serums obtained one and two days after treatment. Necropsy three weeks later revealed that two of the three treated pigs had chronic fibrinous peritonitis. The kidneys of all three treated animals were mottled. The livers appeared normal but, in one pig, there was an abscess between the liver and spleen. The peritoneal surface was covered with young granulation tissue and the kidneys showed evidence of toxic tubular nephrosis.

The reaction after administration of DVE/MA to the Group 2 pigs was severe. The pigs appeared normal immediately after treatment but all three had fever the next day (rectal temperature 103.8 to 104.6°F). The two pigs given the higher doses were extremely lethargic. All three treated pigs had signs of abdominal pain and refused food. Two days after treatment the pig given the highest dose of DVE/MA was found dead. Necropsy revealed severe peritonitis. The pig given 200 mg/kg of DVE/MA was still febrile and was necropsied the same day along with two control pigs. The treated pig had severe peritonitis with strands of fibrin and excess blood-tinged fluid in the abdomen. Neither of the control pigs had gross lesions. Their temperatures and appetites had remained normal. The surviving treated pig remained febrile and inappetent and was killed along with the remaining control pig five days after DVE/MA treatment. The treated pig had severe fibrinous peritonitis. The kidneys were mottled and there was fibrinous pleuritis on one side. The control pig was normal in all respects. Examination of tissues from the treated pigs confirmed the presence of degenerative changes in the kidneys. None of the Group 2 pigs were exposed to FMDV.

Observations After FMDV Exposure

The Group 1 pigs were given 10^4 PFU of FMDV intranasally two days after treatment. There was no difference in time of onset or severity of disease between treated and untreated pigs. Onset and duration of viremia were also the same.

DISCUSSION

Although serum interferon was detected in cattle one hour after single injections of poly (rI.rC), the experiments can be considered negative as to protection against FMDV infection. Cattle given virus by the IDL or IM route two to six hours after being given poly (rI.rC) did not respond differently than untreated control animals. Similarly, goats were not protected from infection after FMDV exposure 24 hours after IV administration of poly (rI.rC). The poly (rI.rC) given the goats had been dissolved in 5% glucose. This would have caused the two strands of the homopolymers (poly rI and poly rC) to separate (9) and negative results might be expected because of the well-established lack of antiviral activity, of single-strand polyribonucleotides. However, from other data (9), when the material was given IV, one might expect that the homopolymers, separated in 5% glucose solution, would come together immediately upon exposure to the ionic environment of the blood stream.

Poly (rI.rC) in doses of 0.3 mg/kg, given IV, protected mice against FMDV exposure (22), whereas doses as high as 4.0 and 5.0 mg/kg did not protect cattle or goats under our conditions. Intraperitoneal administration of 10 mg/kg of IAA (4) or DVE/MA (23) protected some mice against 100 LD_{50} of FMDV, but doses up to 20 times higher failed to protect pigs against contact exposure. The treatment levels may still have been too low. Rosenquist and Loan (24) have observed that when giving a nonreplicating virus to calves, 160 times the effective mouse dose was required to stimulate detectable serum interferon. Serum interferon and protection are not always correlated, however. In mice, protection against virus exposure has been demonstrated with doses of inducer considerably below those required to produce detectable serum interferon levels (5, 20). Furthermore, protection frequently outlasts the presence of interferon in the serum (7, 15, 25) and pigs treated with COAM have experienced a delay in onset of signs of infection with hog cholera virus, yet without detectable interferon activity in the serum (12). Thus, other mechanisms of protection cannot be excluded, although a correlation of dose of poly (rI.rC) serum interferon titer, and ability to protect mice against several viruses (20, 22) support the assumption that poly (rI.rC) is effective against such viruses because of its ability to induce interferon. Protection against herpes simplex virus in mice has been achieved with both poly (rA.rU) and poly (rI.rC) probably by additional protective mechanisms (8) since poly (rA.rU) is a relatively poor inducer of interferon (20).

Our experiments show, as suggested elsewhere (12), that demonstration of protection in an unnatural host (in this case mice) does not predict effectiveness in a natural host. Perhaps, for maximum effect, the interferon or other protective material induced should reach the cells at the place of initial virus multiplication if the infection is to be delayed or aborted, or at the predilection sites to alter the course of the disease. Quite likely the pathogenesis of FMD in suckling mice inoculated intraperitoneally is quite different from that of FMD in cattle, goats or swine after tongue injection or upper respiratory exposure. Since FMDV has been shown to first multiply in the pharynx of cattle and goats after upper respiratory exposure (16, 18, 29) nebulization of the IN-applied poly (rI.rC) may have altered the experiment. Nebulization was used by Angulo and Savan (2) in producing measurable interferon titers in the nasal mucus of calves. Protection at this level of interferon was not tested, however. Therefore, the effectiveness of the treatment against subsequent infection is unknown.

The pyrogenicity of poly (rI.rC) given IV, previously reported for calves (26), rabbits (13, 21), and dogs (21), is now extended to adult cattle and goats. In addition, toxic effects of this complex were observed in goats. A similar transient depression and increased respiration rate have been seen in calves (26). The goat, which died suddenly, and those necropsied three weeks after treatment had evidence of liver degeneration like the findings in dogs by Philips *et al* (21). In all treated goats, the villi of the jejunum were ex-

tensively denuded of epithelium. In view of findings of Philips *et al* (21) on the damaging effects of parenterally-administered poly (rI.rC) on the intestinal lining of mice and rats, and the fact that some of the treated goats developed diarrhea, the complex seems a possible cause. The abortion soon after treatment cannot be proved to have been caused by the poly (rI.rC), but there is a report of embryotoxicity of this complex in rabbits (1).

The IAA appeared to be the least toxic of the three compounds tested. There were no abnormal signs after administration. However, tissues from the treated pigs were not examined. Intraperitoneally administered DVE/MA in pigs led to serious damage. Sellers *et al* (27) have reported evidence of irritation in the peritoneal cavities of similarly-treated cattle and pigs. Toxicity was not unexpected in view of this and other reports (11, 21, 28).

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