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Source: Journal of Wildlife Diseases, 24(3) : 484-490

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-24.3.484

Journal of Wildlife Diseases, 24(3), 1988, pp. 484–490

EFFECT OF INTERFERONS ON THE REPLICATION OF ALCELAPHINE HERPESVIRUS-1 OF MALIGNANT CATARRHAL FEVER

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ABSTRACT: Four isolates of alcelaphine herpesvirus-1 of malignant catarrhal fever (MCF) were tested for their inducibility of and sensitivity to various interferons. Viral isolates from an Indian gaur (*Bos gaurus*), a greater kudu (*Tragelaphus strepsiceros*) and two wildebeest (*Connochaetes gnou*) calves did not induce measurable interferon (IFN) in bovine fetal kidney cells. However, these low passages of each virus were all highly cell-associated and viral replication was inhibited at these passages by IFN at 14 IFN units/0.05 ml recovered from NDV-infected MDBK cells and at 7.6 IFN units/0.05 ml of IFN from NDV-infected bovine macrophages. The herpesvirus from the Indian gaur and greater kudu and high passages (>50) of the cell-free WC-11 strain of alcelaphine herpesvirus-1 also were inhibited in their replication by recombinant IFN of bovine and human origins as determined by a fluorescent focus unit (FFU) reduction assay. The concentrations of IFN required to produce a 50% reduction in herpesvirus-produced FFU ranged between 6.4 and 480 IFN units. These findings promote the use of IFN as part of the regimens of treatment of captive endangered ruminant species with clinical MCF.

Key words: Interferons, alcelaphine herpesvirus, sensitivity testing, Indian gaur, greater kudu, wildebeest, *Bos gaurus*, *Tragelaphus strepsiceros*, *Connochaetes gnou*, experimental study.

INTRODUCTION

Malignant catarrhal fever (MCF) is a fatal lymphoproliferative disease of cattle, deer and exotic ruminant species (Plowright et al., 1960; Plowright, 1981; Heuschele and Castro, 1985) and has been reported to be associated with tumors in a Formosan sika deer (Heuschele et al., 1985). The etiological agent of MCF has been termed bovine herpesvirus-3 (Ludwig, 1983); however, recently a more appropriate classification as alcelaphine herpesvirus-1 has been proposed (Roizman, 1982; Castro and Heuschele, 1985). The herpesvirus of MCF has certain biological characteristics which are similar to the herpesviruses of Epstein-Barr, Marek's disease and herpesvirus saimiri and ateles (Hunt and Billup, 1979). Each of these herpesviruses is classified within the Gammaherpesvirinae subfamily which as a group are primarily lymphotropic, are highly cell-associated, usually produce latent infections in their natural hosts, and may contain oncogenic properties (Hunt and Billup, 1979; Roizman, 1982).

In cattle of Africa, MCF occurs during the calving season of wildebeest. During this calving period, cell-free infectious virus is shed in nasal and ocular secretions of wildebeest neonates and from calves up to 3 mo of age (Plowright, 1965; Mushi et al., 1980). Adult wildebeest do not shed cell-free virus; however, virus neutralizing (VN) antibody is present throughout their lives (Plowright, 1967). The isolation of alcelaphine herpesvirus-1 from clinical MCF is accomplished by either direct cultivation of trypsinized cells from specific organs (spleen, lung, lymph nodes, thymus) or by co-cultivation of buffy coat cells with bovine fetal kidney (BFK) or thyroid cells (Plowright et al., 1960).

Although herpesviruses have been considered relatively unaffected by the effects of IFN and herpesviruses are poor inducers of IFN, experimental evidence recently has indicated that specific herpesviruses

are affected by IFN and may induce IFN production (Vaczi et al., 1965/1966; Libikova, 1973; Schat et al., 1974; Lvovsky et al., 1981; Letchworth and Carmichael, 1983; Fulton and Burge, 1985). Investigations (Todd et al., 1971, 1972) with avirulent infectious bovine rhinotracheitis (IBR) virus or BHV-1 have shown that IFN was induced in calves when IBR virus was given intranasally at 72 to 96 hr prior to challenge by virulent IBR virus and these calves became refractory to disease. Although, the avirulent virus had to be given at a specific time and the effect of IFN was transient, induced IFN persisted for a 10-day interval. Experiments using cell cultures have demonstrated that endogenous IFN does protect cells against infection by Epstein-Barr virus (Lvovsky et al., 1981), Marek's disease virus (Schat et al., 1974) and pseudorabies virus (Libikova, 1973). However, persistent infections in cell cultures infected with herpesvirus (Glasgow and Habel, 1963) have been produced by interferon-like substances in the absence of specific antibody.

There is a paucity of information on the interaction between alcelaphine herpesvirus-1 with IFN; therefore, the objectives of this study were to determine whether isolates of alcelaphine herpesvirus-1 were inhibited in their replication in cell cultures by homologous and heterologous IFN and if isolates of alcelaphine herpesvirus-1 were inducers of IFN.

MATERIALS AND METHODS

Cells and viruses

Four viruses identified as alcelaphine herpesvirus-1 which had been isolated from an Indian gaur (*Bos gaurus*), a greater kudu (*Tragelaphus strepsiceros*) (Castro et al., 1981) and two wildebeest calves (*Connochaetes gnou*) (Castro et al., 1984) were propagated in primary BFK cells as described previously (Castro et al., 1981). Serial passages of each virus were done by co-cultivation of cells infected with each virus with healthy BFK cells (Castro et al., 1981, 1984). Viral cytopathology as measured by formation of syncytia was used as an indicator of the replication of each of the four herpesviruses. The prototype WC-11 virus was propagated in bovine turbinate (BT) cells (kindly provided by C. Metz of the National Veterinary Service Laboratories, Ames, Iowa 50010, USA) and harvested by three cycles of freeze and thaw of the virus-infected cells. Cultures of BFK and BT cells were grown and maintained in Dulbecco's growth medium containing 10% fetal bovine serum and 200 μg/ml of gentamicin (GIBCO Laboratories, Grand Island, New York 14072, USA).

Assay for IFN

Culture fluids were harvested at varying intervals between 0 and 120 hr from cell monolayers infected with each of the four herpesvirus isolates. These cell-free fluids were diluted 1:5 (v/v) in growth medium and 0.5 ml of each harvest was inoculated into duplicate wells of a 24-well plate containing confluent 48-hr monolayers of BFK cells. The inocula of culture fluids were allowed to adsorb onto the cell monolayers for 24 hr at 37 C, removed by aspiration, and then washed once with Dulbecco's medium. The level of IFN of each harvest was determined by the IFN assay previously described by Epstein (1976). For the IFN assay, the Indiana strain of vesicular stomatitis virus (VSV) (Epstein, 1976) was used at challenge concentration of 40 to 60 plaque forming units (PFU)/well. Uninfected culture fluids from BFK cells were collected at the same intervals as controls. The titers of IFN in the harvested fluids for each virus were calculated by the method described by Epstein (1976).

Types of IFN

The IFN used in this investigation were of bovine (BoIFN) and human (HuIFN) origins. The BoIFN were previously prepared by the infection of either a cell line of bovine kidney (MDBK) or bovine alveolar macrophages (BAM) with Newcastle disease virus (NDV): these bovine derived IFN were labeled BoIFN-NDV-MDBK and BoIFN-NDV-BAM, respectively. The characteristics and the method of preparation of the BoIFN-NDV-MDBK and BoIFN-NDV-BAM have been described by Fulton and Burge (1985). The BoIFN-α1 (Genentech Inc., San Francisco, California 94080, USA) and HuIFN-αA/D (Hoffmman LaRoche, Netley, New Jersey 07110, USA) were prepared in cultures of *Escherichia coli* by recombinant DNA technology.

The IFN units of BoIFN-NDV-MDBK and BoIFN-NDV-BAM and both commercial recombinant IFN had been determined prior to the experiment. Briefly, to assay for IFN activity, 0.05 ml of serial dilutions of each of the four IFNs were added to confluent monolayers

TABLE 1. Effect of two different interferons on the replication of alcelaphine herpesvirus-1 in BFK cells.

Alcelaphine herpes viruses tested	Interferons tested: BoIFN-NDV-MDBK[a] Fluorescent focus unit (FFU) in BFK: IFN treated	BoIFN-NDV-MDBK[a] Fluorescent focus unit (FFU) in BFK: Untreated	BoIFN-NDV-MDBK[a] % Reduction in FFU	BoIFN-NDV-BAM[b] Fluorescent focus unit (FFU) in BFK: IFN treated	BoIFN-NDV-BAM[b] Fluorescent focus unit (FFU) in BFK: Untreated	BoIFN-NDV-BAM[b] % Reduction in FFU
Gaur	43.5[c]	231.2	81.2	40.5	204.5	80.2
Greater kudu	263.5	697.0	62.2	314.5	640.0	50.9
Wildebeest 1	54.0	227.5	76.3	89.5	240.0	62.7
Wildebeest 2	119.0	426.5	72.1	199.0	442.0	55.0

[a] 14 IFN units/0.05 ml/well.
[b] 7.6 IFN units/0.05 ml/well.
[c] Mean of two IFN assays.

of BFK cells and adsorbed for 2 hr at 37 C. Then, 1 ml of growth medium was added to each well. After a 24-hr incubation at 37 C, cells were rinsed once with medium and challenged with VSV. To ascertain possible toxicity of each IFN, cells treated with each IFN served as controls. Because of limited amounts of available BoIFN-NDV-MDBK and BoIFN-NDV-BAM, a single concentration of each of these IFN's was used to determine the activities of the IFN to each isolate. To determine the minimal units of recombinant IFN that produced a 50% reduction in virus produced fluorescent foci, 10-fold dilutions of the two available recombinant IFNs were used.

Assay of IFN by reduction of virus produced fluorescent focus unit

Because the cell passages were <20 of the four alcelaphine herpesviruses each of which were mostly cell-associated, the activity of each IFN on each alcelaphine herpesvirus was done by counting virus-produced fluorescent foci in BFK and BT cells. Each focus of viral fluorescence (nuclear and cytoplasmic) was designed as a fluorescent focus unit (FFU). By this method, comparisons of cells infected with each of the four isolates of alcelaphine herpesvirus to virus-infected cells treated with IFN were done. The activity of each IFN for each virus was expressed as the percent reduction of FFU of IFN-treated and non-treated virus infected cell monolayers.

To determine the sensitivity of the WC-11, Indian gaur, greater kudu and two wildebeest isolates of alcelaphine herpesvirus-1 to varying concentrations of different IFN, cell monolayers of BFK or BT infected with each virus were trypsinized with 0.05% trypsin (GIBCO Laboratories, Grand Island, New York 14072, USA) and then dispersed as single cells into growth medium. Cells containing each virus were then dispensed at 0.6 ml/well into a 4-chamber Lab-Tek slide (Arthur H. Thomas Co., Philadelphia, Pennsylvania 19105, USA). A 0.05 ml inoculum containing either 14 units of BoIFN-NDV-MDBK or 7.6 IFN units of BoIFN-NDV-BAM was simultaneously added into duplicate wells. Subsequently, the IFN-treated cells were incubated at 37 C in a humidified CO_2 incubator for 7 days. Following incubation, cell monolayers on each slide were washed three times with 0.01 M phosphate buffered saline, pH 7.2 (Sigma Chemical Co., Saint Louis, Missouri 63178, USA), fixed for 10 min in cold acetone and rinsed in distilled water. The fixed slides were air-dried and stored at −70 C until used for the indirect immunofluorescent (IIF) assay. The recombinant IFN were tested similarly using serial 10-fold dilutions.

The antiserum to alcelaphine herpesvirus-1 used in the IIF assay was obtained from a gaur which died with clinical MCF (Castro et al., 1981). A rabbit antibovine immunoglobulin G (IgG) fluorescein-conjugate used in the IIF was obtained commercially (Miles Laboratories, Inc., Naperville, Illinois 60566, USA). The methodology used for the fluorescent staining and detection of immunofluorescent cells infected with the alcelaphine herpesvirus has been described (Castro et al., 1984).

RESULTS

In duplicate assays, IFN activity was not detected in fluids from each virus as measured by a ≥50% reduction in PFU of VSV. However, a reduction in PFU of VSV of <10% was measured in the supernatant fluids collected at 96 to 120 hr from cell cultures infected with the wildebeest-1 viral isolate. In the IFN assays, the fetal bo-

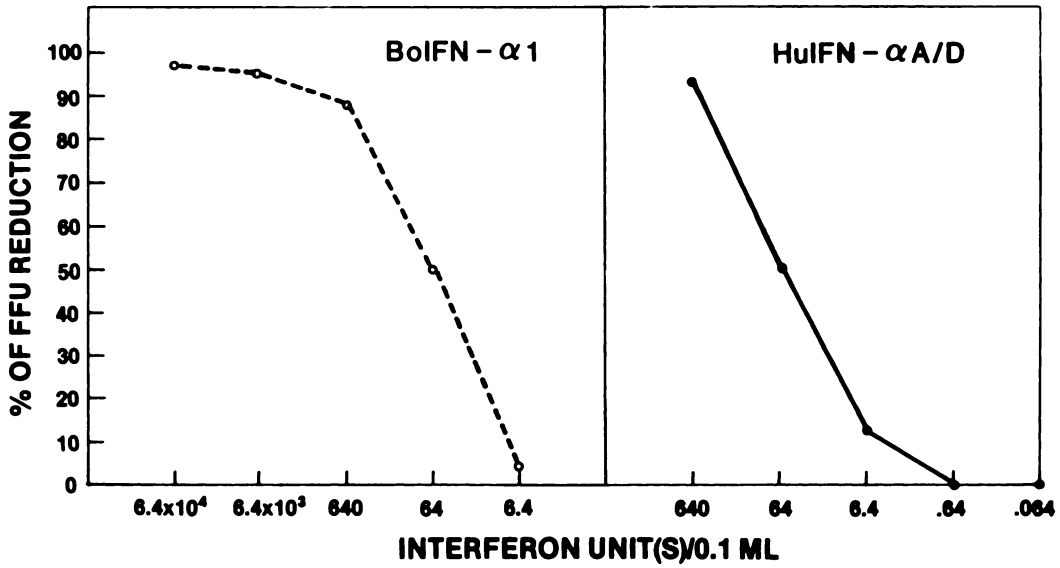

FIGURE 1. Effect of bovine and human recombinant interferons on the reduction of fluorescent focus formation in BT cells infected with WC-11 strain of alcelaphine herpesvirus-1. The input virus titer was 430 fluorescent focus units/well.

vine serum used had previously tested negative for the presence of adventitious agents and endogenous IFN. The uninfected cell cultures also were tested negative for endogenous IFN.

The effects of IFN of different origins (BoIFN-NDV-MDBK and BoIFN-NDV-BAM) on the replication of each isolate of alcelaphine herpesvirus-1 in BFK cells are presented in Table 1. For viral isolates tested, a reduction between 62 and 81% in FFU was found after treatment of cells with 14 IFN units of BoIFN-NDV-MDBK. Reductions in FFU for the four viruses from between 50 and 80% were found after treatment of cells with 7.6 IFN units of BoIFN-NDV-BAM (Table 1).

The activities of the two IFNs produced by recombinant DNA technology against three of the alcelaphine herpesviruses also were compared. Dilutions of homologous (bovine) BoIFN-α1 and a heterologous (human) HuIFN-αA/D were assayed against the prototype WC-11, Indian gaur and greater kudu isolates of alcelaphine herpesvirus-1. At the dilution which contained 64 IFN units of either BoIFN-α1 or HuIFN-αA/D, a 50% reduction in FFU occurred in BT cells infected with the WC-11 strain (Fig. 1). A 50% reduction in FFU also occurred when BFK cells infected with the greater kudu strain were treated with 64 IFN of BoIFN-α1. However, 480 IFN units of HuIFN-αA/D were required to produce a 50% reduction in FFU in BFK cells infected by the greater kudu strain of virus (Fig. 2). In BFK cells infected with the Indian gaur isolate, a concentration of 6.4 IFN units of either BoIFN-α1 or HuIFN-αA/D produced a 50% decrease in FFU units (Fig. 3).

DISCUSSION

Replication of each of the four isolates of alcelaphine herpesvirus-1 in cell cultures was found to be partially inhibited by each IFN tested; however, the level of inhibition of virus replication varied between viral isolates. Although the reduction in FFU for each alcelaphine herpesvirus following treatment by BoIFN-NDV-MDBK was slightly greater than BoIFN-NDV-BAM, the units of IFN of BoIFN-NDV-BAM were half of those used for BoIFN-NDV-MDBK. This finding suggests that the replication of each of the four alcelaphine herpesviruses was more sensitive to IFN produced in bovine al-

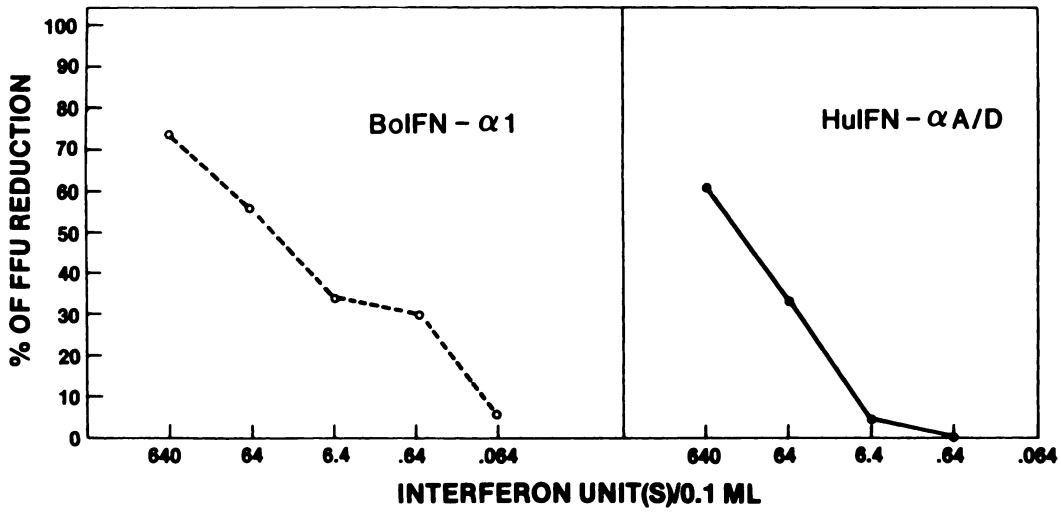

FIGURE 2. Effect of bovine and human recombinant interferons on the reduction of fluorescent focus formation in BFK cells infected with the greater kudu isolate of alcelaphine herpesvirus-1. The input virus titer was 669 fluorescent focus units/well.

veolar macrophages than to IFN induced from a bovine cell line.

Low cell culture passages of each of the viral isolates of alcelaphine herpesvirus-1 did not induce measurable levels of IFN in BFK cells. Nevertheless, the replication of each of the four herpesviruses in cell cultures was significantly inhibited by homologous (bovine) cell culture derived IFN and also by homologous (bovine) and heterologous (human) recombinant IFN.

The replication of a high cell culture passage of cell-free alcelaphine herpesvirus (WC-11 strain) was sensitive also to IFN similar to the sensitivity to IFN by four cell-associated herpesviruses present at low cell passages. In contrast, the cell-free murine cytomegalovirus, a herpesvirus, was found to be susceptible to inhibition by IFN but virus that was cell-associated was not affected (Oie et al., 1975).

Although the inhibition of replication of alcelaphine herpesviruses in vitro by these IFNs was encouraging, this finding cannot presently be equated to the possible effects in vivo of these IFN. Effective methods of treatment of MCF in susceptible, captive ruminants is non-existent. Nonetheless, the emergency treatment of clinical MCF in a captive and endangered exotic ruminant species with either bovine or human-derived recombinant IFN should be a consideration in animals at risk.

The present recommended management of MCF in ruminants in zoological gardens is a program of non-breeding of wildebeest herds (Castro and Heuschele, 1985; Heuschele and Castro, 1985). This technique has reduced the incidence of transmission of MCF to susceptible and endangered species such as the Pere David's deer (*Elaphurus davidianus*) (Wan and Castro, unpubl. data). But, the possibility of certain exotic breeds of sheep such as mouflon (*Ovis musimon*) carrying this herpesvirus in a defective form poses a threat to captive and free-roaming herds of wildlife within the United States and Europe. In England, Reid et al. (1979) have reported on major clinical outbreaks of sheep-associated MCF in red deer (*Cervus elaphus*), a common deer species in Europe.

Although intensive symptomatic, supportive and antibiotic treatment has been widely used in MCF-affected endangered captive ruminant species, such measures have been unsuccessful in the prevention of the death of animals. The effective in

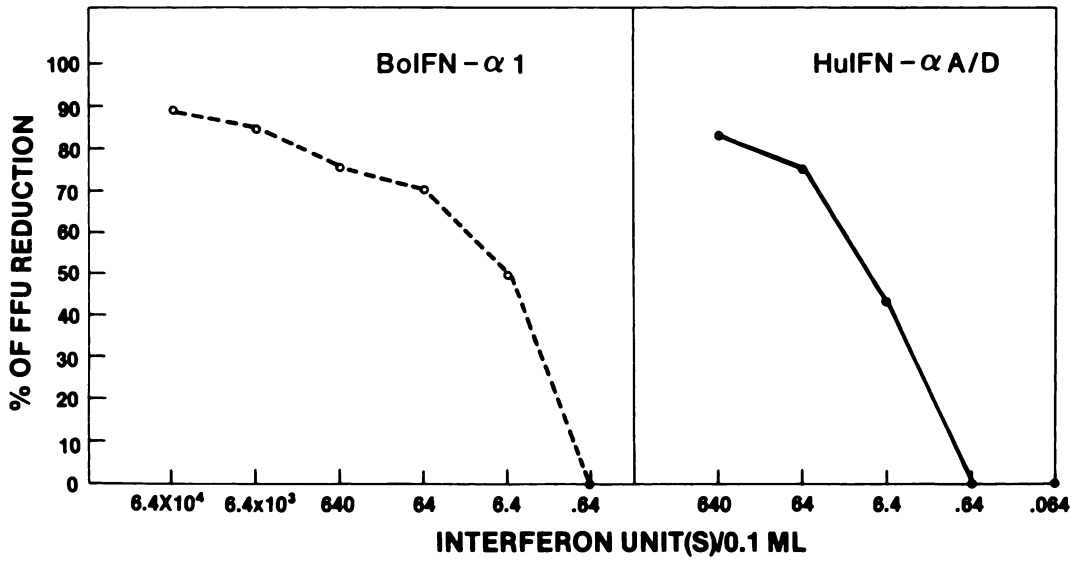

FIGURE 3. Effect of bovine and human recombinant interferons on the reduction of fluorescent focus formation in BFK cells infected with the Indian gaur isolate of alcelaphine herpesvirus-1. The input virus titer was 211 fluorescent focus units/well.

vitro inhibition of virus replication of four field isolates of alcelaphine herpesvirus offers an additional approach in the treatment regimen for captive ruminants which may be exposed to this virus. Treatment with IFN or inducers of IFN may decrease the severity of an infection by the alcelaphine herpesvirus and may save rare species which are potentially on the verge of extinction.

Because MCF may be lethal to the infected host, adequate control and prevention must await the development of a subunit vaccine and effective methods (DNA probes) of diagnosing carrier species. Therefore, an appropriate regimen of supportive therapy could include the use of synthetic IFN or inducers of IFN to species clinically affected with MCF. However, if an animal infected with alcelaphine herpesvirus survives, it may become persistently infected with the virus. Thus, this animal may pose a threat to susceptible species with which it contacts. Nonetheless, if the captive species is threatened or endangered, the survival of the species may outweigh a latent infection with an alcelaphine herpesvirus. In these cases, breeding should be restricted, carriers must be identified and such animals need to be removed from the proximity of susceptible species.

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Received for publication 26 September 1986.