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Natural Human Interferon-α Administered Orally as a Treatment of Bovine Respiratory Disease Complex

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ABSTRACT

Natural human interferon-α (nHuIFN-α) from three sources was given orally to 368 calves experiencing a natural outbreak of bovine respiratory disease complex (BRDC). In one study, 200 calves were given one treatment daily for 3 days of placebo or 20, 200, or 2,000 IU of nHuIFN-α before shipment. Calves treated with 20 or 200 IU had a significant ($p < 0.05$) weight gain benefit for the first 21 days in the feedlot, if they had rectal temperatures $<40°C$ when treated with nHuIFN-α. In a second trial, 168 mixed-breed calves (five groups randomized to 31–36 calves/group) were treated with one dose of placebo or 200 or 400 IU of nHuIFN-α after shipment to the feedlot. Using this regimen, a dose of 200 IU per calf significantly ($p < 0.08$) decreased the number of sick calves per group and delayed development of BRDC. Results of these studies demonstrate that oral administration of nHuIFN-α, which may partially mimic the nasally secreted IFN response reported during BRDC, may be beneficial in cattle.

INTRODUCTION

BOVINE RESPIRATORY DISEASE COMPLEX (BRDC), or shipping fever, causes the greatest morbidity and mortality of any infectious disease complex in the United States cattle industry.[1,2] BRDC is a broadly applied term used to denote an acute, highly contagious mixed infectious disease of young stressed cattle characterized by fever ($>40°C$), inflammation of the upper respiratory tract, bacterial and/or viral pneumonia with clinical signs of dyspnea, anorexia, fever, depression, and mucopurulent ocular and nasal discharges. The BRDC accounts for the majority of feedlot losses, and are the primary cause of death in the feedlot. Of cattle that develop BRDC, most develop signs within 14 days of entry into the feedlot.[1,2]

BRDC is primarily a result of modern production and management practices. Young post-weaning beef cattle are most susceptible to disease and develop BRDC during the processes of collection, sorting, branding, ear tagging, deworming, vaccinating, delousing, and dehorning that commonly occur prior to shipment to sale barns and feedlots. These cattle are also subjected to the stresses of crowding, abrupt changes in diet and environment, lengthy transportation without food or water, and exposure to viral and bacterial respiratory pathogens.[1,2]

The infectious agents encountered by calves upon entering into the marketing system are many and varied. Viruses implicated in the pathogenesis of BRDC include infectious bovine rhinotracheitis (IBR) virus, parainfluenza type 3 virus, bovine viral diarrhea (BVD) virus, respiratory syncytial virus (RSV), and many serotypes of adeno-, entero-, rhino-, parvo-, and reoviruses. Bacterial pathogens (chiefly the *Pasteurella* spp., *Hemophilus somnus*, and *Mycoplasma* spp.) colonize virus-devitalized respiratory tracts and are responsible for the more severe clinical manifestations of BRDC.[3]

Containment of the BRDC complex is problematic. Preventive measures such as vaccination against the various infections, mass medication with antibiotics, and preconditioning may reduce the severity of BRDC but in general are not successful in prevention. Furthermore, these activities are expensive, labor-intensive, and may actually induce BRDC. Other methods for control of BRDC are needed by the feedlot industry.

Prominent in the disease complex is IBR virus infection, a highly contagious bovine herpesvirus infection of the upper res-

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piratory tract.[4] Vaccination for IBR is of limited benefit because the mechanics of cattle handling and transport promote development of naturally occurring BRDC during the first two weeks in the feedlot. The IBR virus is a potent *in vivo* inducer of IFN[5,6] if given systematically, and IFN-like activity has been described in the nasal secretions (NS) of cattle after intranasal administration of IBR vaccine or virulent viruses.[7–12] The NS-IFN may be of benefit in reducing manifestations of IBR and BRDC. Here, we report the use of varying low doses of nHuIFN-α either prior to, or after, shipment on the reduction of the clinical manifestations of BRDC in feedlot cattle.

Treatment of BRDC with nHuIFN-α

Three sources of nHuIFN-α were evaluated for efficacy in the feedlot trials described below. For trial 1, nHuIFN-α was produced by the Cantell method[13] by Immune Modulators Laboratories (IML; Stafford, TX). Stock nHuIFN-α was diluted in phosphate-buffer ed saline (PBS) to concentrations of 20, 200, or 2,000 IU of IML nHuIFN-α/calf. For trial 2, doses of 200 or 400 IU/calf of nHuIFN-α were supplied by Hayashibara Biochemical Laboratories, Inc. (HBL; of Okayama, Japan) and also by Interferon Sciences, Inc. (ISI; New Brunswick, NJ).

Cattle for feedlot trials

In the first trial, 200 mixed breed calves were purchased from six auction barns in Kingsport, Greenville, Sweetwater, Knoxville, Morristown, and Telford, Tennessee, using an order buyer in Newport, Tennessee. The purchase weights ranged from 370 to 545 (mean 461) lbs. These calves were commingled for 3 days and then shipped (24 h, 1,180 mi) by truck to the Texas A&M University Research Center, Amarillo, TX, where they remained for 46 days. Calves were assigned to treatment groups of 50 by weight and sale of origin and these were randomly allotted to treatment groups. In the second trial, 200 mixed breed calves (mean weight 418 lbs) were purchased from a Texas order buyer and shipped to the same Texas location and were on test for 14 days. The cattle were sorted into 5 groups of 40 each by weight. Calves with rectal temperatures $\geq$40°C (n = 32) were judged to be clinically ill prior to entry into the therapy trial and excluded from the analysis of efficacy of nHuIFN-α treatment.

Experimental design for nHuIFN-α therapy

For feedlot trial 1, calf groups were given nHuIFN-α (0, 20, 200, or 2,000 IU) orally once daily for 3 consecutive days in Tennessee. Nasal secretions for determination of NS-IFN were collected from each calf before the first treatment. Serum for viral serology was collected from each calf 1 and 28 days after arrival in Texas. Calves were individually weighed on arrival and again at 11, 14, 21, 28, and 46 days after arrival. Mean daily weight gains were calculated from the pay weights recorded at the auction barns of origin. Rectal temperatures were individually determined by an electronic thermometer daily for 3 consecutive days when nHuIFN-α treatment was given in Tennessee and again upon arrival in Texas, during processing, and during antibiotic treatment when calves were scored for BRDC (see Clinical Evaluations). Daily feed consumption was determined for individual calves using an automatic feed monitoring device (Pinpointer®, Cookeville, TN).

In feedlot trial 2, a randomized block design for placebo (0 IU) and four treatment groups (200 and 400 IU of HBL nHuIFN-α and 200 and 400 IU of ISI nHuIFN-α) was used. Calves were blocked by weight with the heaviest to the lightest animal forming blocks of 5 each. The treatments upon arrival were blinded by group and delivered to investigators in individual dose syringes containing 4 ml each and labeled 1 through 200. Cattle were weighed and ear tagged upon arrival and assigned to pens (20 calves/pen). Within the blocks, the five treatment regimens (one treatment on arrival) were randomly allotted using a random number table. The placebo-treated control calves were randomly allotted in blocks of 2 between the HBL and ISI diluent, without nHuIFN-α.

Clinical evaluations

In study 1, calves were observed daily for clinical signs of BRDC and judged by the following weighted scoring system for degree of clinical illness as defined by the scale shown in Table 1. A calf with a score of $\geq$5 was considered clinically ill and treated with antibiotics (erythromycin or oxytetracycline). In study 2, calves with a mucopurulent nasal discharge and rectal temperature of $\geq$40°C were judged clinically ill with BRDC and were treated with a broad spectrum antibiotic (Micotil®). Calves with diarrhea, but not BRDC, were treated for enteric infection with orally administered neomycin sulfate at the recommended dose. For both studies, calves that died during the study were transported to the Texas Veterinary Diagnostic Laboratory (Amarillo, TX) for a complete necropsy and determination of the cause of death.

Statistical evaluation of the data

In feedlot trial 1, analysis of variance (ANOVA) and Chi square techniques were applied to experimental data; p values of $<$0.10 were considered statistically significant. In feedlot trial 2, any animal treated with antibiotics was considered to be sick and constituted an IFN-α treatment failure. The number of sick calves within treatment groups were subjected to Chi square analysis. Calves that were ill at the time of the first IFN-α treatment were judged to have illness prior to the study and were deleted from the treatment efficacy analysis. Rectal temperatures, weights, mean daily weight gains, feed intake, and feed:gain ratios were collected and analyzed by Chi square and ANOVA techniques.[14] Means were separated by Duncans Multiple Range Test.

TABLE 1. SCORING SYSTEM FOR DEGREE OF CLINICAL ILLNESS

Scoring system for illness (variable)	*Points*
Anorexia (<0.1 lbs intake/day)	3
Anorexia (0.2–0.9 lbs intake/day)	2
Fever ≥41.1°C	3
Fever ≥40–41°C	2
Fever ≥39.4–39.9°C	1
Depression or cough, each	1
Purulent nasal or ocular discharge, each	1
Maximum possible score	10

RESULTS

Feedlot trial 1: IML IFN-α as a therapy for BRDC

At the time of arrival in Texas, more than half of the calves were seronegative to PI-3, BVD, IBR, and RSV viruses. Seroconversion to PI-3, RSV, and BVD viruses but not IBR virus was widespread in every treatment group.

Forty-five percent (89 of 200) of the cattle enrolled had detectable NS-IFN prior to treatment. The NS-IFN-positive calves were randomly distributed within each treatment group. The incidence of BRDC illness was significantly ($p < 0.05$) reduced by 20 IU of nHuIFN-α therapy compared to calves given 2,000 IU (66% versus 84%, respectively).

Calves with NS-IFN at the time of the nHuIFN-α treatment may have responded differently to nHuIFN-α therapy than calves without detectable NS-IFN. At 46 days, a weight gain benefit ($p < 0.01$) occurred in calves with pre-existing NS-IFN titers > 100 units when given 20 IU nHuIFN-α, compared to placebo.

The effects of nHuIFN-α treatment upon feed conversion, as measured by feed-to-gain (F/G) ratios, were determined. In calves with BRDC, the F/G ratio was significantly ($p < 0.10$) better if calves were given 20 IU, compared to placebo (9.3 lbs of feed/lb of gain compared to 13.6 lbs of feed/lb of gain). In other words, calves given 20 IU of nHuIFNα gained more weight on less feed, presumably because the BRDC was less severe in these nHuIFNα treated cattle.

When all cattle were compared, feed intake was not significantly different between treatment groups. However, calves without fever, treated in Tennessee with either 20 or 200 IU nHuIFN-α gained significantly more weight for 21 and 28 days after arrival in Texas (Table 2).

Feedlot trial 2: HBL IFN-α and ISI IFN-α as therapy for BRDC

Two hundred calves were received; 32 were removed from the study upon arrival because of fever >40°C. The remaining 168 calves were divided into groups of 31–36 individuals as shown in Table 3.

TABLE 2. THE EFFECT OF nHuIFN-α, ADMINISTERED ORALLY, ON MEAN DAILY WEIGHT GAINS (MDG) AT 21 AND 28 DAYS AFTER ARRIVAL (CALVES WITH RECTAL TEMPERATURES <40°C AT THE TIME OF TREATMENT BEFORE SHIPMENT)

nHuIFN-α dose (IU/calf)	*Number of calves*	*MDG by day*[a]	
		21	*28*
0	20	0.24[b]	0.97[b,c]
20	25	1.11[c]	1.73[b]
200	20	1.25[c]	1.70[b]
2000	24	0.61[b,c]	0.85[c]

[a]Pounds of weight gain.

[b,c,bc]Numbers with superscript[b] are significantly ($p < 0.05$) different from numbers with superscript[c] in same column; numbers with superscript[b,c] are not significantly different from numbers with either superscript[b] or superscript[c].

TABLE 3. EFFECT OF nHuIFN-α, ADMINISTERED ORALLY ON THE PERCENTAGE OF CALVES TREATED FOR BRD

Source and dose (IU) of nHuIFN-α	*Number of calves*	
	Total	*Treated (%)*
HBL (200)	36	19 (53)
HBL (400)	34	20 (59)
ISI (200)	33	15 (45)[a]
ISI (400)	34	23 (68)
Placebo	31	23 (74)

[a]Significantly different from placebo at $p < 0.08$.

Twenty-three of 31 (74%) control calves required antibiotic treatment for BRDC. In contrast, calves treated with 200 IU ISI nHuIFN-α had significantly ($p < 0.08$) fewer morbid calves (15 of 33, 45%). Similarly, the percent of calves (53%) requiring antibiotic treatments after 200 IU HBL nHuIFN-α therapy was reduced and nearly significant ($p = 0.12$), compared to controls. Therapy with 400 IU of HBL or ISI nHuIFN-α did not significantly reduce morbidity (Table 3).

The nHuIFN-α administered orally did not delay the onset of BRDC. There were no significant differences in body weights, mean daily weight gains, feed intake, or F/G ratios between IFN-α treatment groups; the calves in this study were observed for only 14 days.

DISCUSSION

The nHuIFN-α as a treatment for BRDC prior to shipment was tested in field trial 1. More than one-half of the calves given nHuIFN-α orally prior to departure to Texas tested seronegative for PI-3, BVD, IBR, and RSV viruses. After transport, seroconversion to PI-3, RSV, and BVD viruses, but not the IBR virus, was widespread in every treatment group (data not shown). BRDC illness was reduced by 20 IU nHuIFN-α therapy compared to 2000 IU, demonstrating that the lower-dose regimen was more efficacious; the phenomenon of "use of less IFN-α orally is better" has been demonstrated previously in cattle[15] and horses.[16]

Calves exhibiting existing NS-IFN titers of >100 units/ml had a weight gain benefit when given 20 IU of nHuIFN-α, compared to controls. The F/G ratio in cattle with BRDC given 20 IU nHuIFN-α was favorable versus controls 46 days after arrival. Calves in the 20 or 200 IU nHuIFN-α treatment group exhibited significantly improved weight gains at 21 and 28 days after arrival (Table 2). The second feedlot study indicated that a single low dose of nHuIFN-α reduced morbidity of calves with BRDC. Both 200 IU doses of nHuIFN-α, when compared to controls, regardless of company origin, showed a benefit in reducing morbidity.

The predominant IFN in the NS of cattle appears to have characteristics of an IFN-α. An important characteristic that NS-IFN shares with IFN-α is its virus-inhibiting properties, which may have important applications for the cattle industry, especially in the treatment of BRDC. As our trials indicate, a single dose of low-dose oral nHuIFN-α was safe and nontoxic,

reduced morbidity, and provided both a favorable F/G ratio and a weight gain benefit. Based upon these results, additional evaluation of nHuIFN-α given orally in feedlot animal production is warranted.

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