A Clinical Trial of Recombinant Bovine Interferon α_1 for the Control of Bovine Respiratory Disease in Calves

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ABSTRACT. The effects of recombinant bovine interferon α_1 (rBoIFNα_1) on the incidence of bovine respiratory disease (BRD) were examined in 60 young male cattle. Each group of clinically healthy 30 male cattle was injected intramuscularly with either rBoIFNα_1 (5 mg/head) or physiological saline 5 times in 6 months. They were examined daily for the clinical symptoms of respiratory distress. The clinical signs of respiratory distress in rBoIFNα_1-treated cattle were significantly less severe than those of the placebo group. The incidence rate, relapse rate and mean sick days of respiratory disease in the rBoIFNα_1-treated group were 23%, 14% and 4.43 days, respectively, whereas those in the placebo group were 80%, 46% and 6.42 days, respectively. Furthermore, the mean increase of body weights in rBoIFNα_1-treated group and placebo group were 1.365 kg/day and 1.340 kg/day, respectively. These results suggest that rBoIFNα_1 has a potentially preventive effect on the incidence of BRD.—KEY WORDS: cattle, immunomodulation, inflammation, interferon, respiratory disease (bovine).

Improvement in the development and delivery of vaccines and chemical drugs has contributed to the control of a wide variety of acute infectious diseases in economically important cattle. However, bovine complex diseases including bovine respiratory disease (BRD), mastitis and diarrhea at the neonatal period continue to present a serious economic burden to the producer. In particular, BRD is a complex disease characterized by inflammation of the respiratory tract and is referred to generally as shipping fever, as occurs most frequently within the first week after moving cattle to feedlots. Cattle with BRD reveal the compromized immune system resulting from many interactions among various stress factors and pathogens [2, 3, 16]. Most of the cattle with BRD recover by conventional chemical treatment with antibiotics, but some may die or remain in chronic BRD, suggesting that the effectiveness of antibiotics in BRD prevention and treatment is limited.

The interferons (IFN), a family of cytokines, have been well known to have a variety of biological effects including antiviral activity and immunomodulating activity. A novel approach by using different recombinant cytokines including IFNs produced by genetic engineering technology to control various diseases has been tried in human and veterinary medicine [4, 5, 9–10, 11, 14, 17], and some promising results have been reported in certain diseases [2, 4, 9].

In the present study, the effects of recombinant bovine IFNα_1 (rBoIFNα_1) on the incidence of BRD and gain of body weight per day in young male cattle were examined to assess a possible means of preventing BRD in natural field situations.

MATERIALS AND METHODS

Cattle: Sixty heads of 7 to 14 days old healthy male calves (mean body weight, 56 kg) were introduced from dairy farms around Ashoro, Hokkaido. Initially, they were kept in individual boxes (calf hutches) for 35–40 days by feeding commercial milk replacer. They were divided into two groups of 30 calves. One group was subjected to rBoIFNα_1 treatment and the other to the placebo control. Subsequently, a herd of 5 to 6 heads in each group was kept together in larger growing (fattening) boxes (super hutches) for 30 days by feeding commercial food including milk replacer and starter. Then, a herd of 30 calves in each group was moved to growing pens and kept there for 12 weeks by feeding corn silage, concentrate and water *ad libitum*.

Interferon: rBoIFNα_1 kindly provided by Ciba Geigy Japan Ltd., Co. (Tokyo, Japan) was used. It was produced by *E. coli* by recombinant DNA technology and prepared as sterile, lyophilized substance for injection in 50 mg vials containing 25 mg protein. The specific activity is $\geq 10^7$ U/mg protein. The vial content was reconstituted with 50 ml sterile water prior to administration. Each cattle in rBoIFNα_1-treatment group was injected intramuscularly with 2 ml of 5 mg rBoIFNα_1 at the treatment schedule shown in Fig. 1, since the dose has been reported to be effective in cattle with experimentally induced BRD [2]. In brief, rBoIFNα_1-treatment was done 2 days prior to moving cattle from individual calf hutches to super hutches (at 7 weeks of age), and then 2 days before setting them from super hutches to growing pens (at 12 weeks of

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age). Furthermore, during keeping at a growing pen for 12 weeks, they were additionally treated three times with the same dose of rBoIFNα_1 at the ages of 16, 18 and 20 weeks, respectively. Each cattle in the placebo control was injected intramuscularly with 2ml of physiological saline without rBoIFNα_1 at the treatment schedule shown in Fig. 1.

Management practices: Close contact between the treatment group and the placebo control group was not provided throughout the present experiment. Cattle diagnosed as apparently clinical respiratory disease received conventional antibiotic therapy by intramuscular injection; initially a combination of penicillin/dihydrostreptomycin (10,000 IU/12.5 mg/kg body weight) and followed by kanamycin (10 mg/kg body weight), if nasal swabs testing by the disc method indicated susceptibility to kanamycin.

Parameters to be evaluated: Cattle were examined daily especially for the clinical signs of respiratory distress including nasal discharge by blinded observers who were not informed of the allocation of animals to treatment and

Fig. 1. Experimental design.

Table 1. Parameters examined for clinical scoring[a]

Parameter	Younger than 3 months	Older than 3 months	Score
Body temperature	<39.5°C	<38.9°C	1
	39.5–40.4°C	38.9–40.2°C	2
	>40.4°C	>40.2°C	3
Appetite/behavior	Normal		1
	Reduced		2
	Anorexia/moribund		3
Cough	None		1
	Infrequent, painless, mild		2
	Frequent, painful, severe		3
Nasal discharge	None		1
	Serous		2
	Mucopurulent		3
Respiratory rate	<40	<30	1
	40–60	30–40	2
	>60	>40	3

a) Cattle were scored daily for clinical symptoms, and the total score for each sick calf was used to calculate a daily mean for each group. More than 2 in daily mean scores was considered to be sick. Coefficients set for body temperature, appetite/behavior, cough, nasal discharge and respiratory rate were 3, 3, 1, 2 and 3, respectively. Mean scores were calculated by dividing total values in clinical scoring by total coefficients, 12.

control groups. Clinical scores for the parameters shown in Table 1 were recorded. Some cattle with clinical symptoms of respiratory disease were randomly subjected to bacterial, virological and serological examinations. Nasal discharge samples taken from sick cattle by using sterile cotton swabs were cultured on 10% horse blood agar plates to isolate and identify *Pastrella multocida, P. hemolytica, Actinomyces pyrogens* and *Hemophilus somnus*. Furthermore, blood samples, collected from some cattle at the both days diagnosed as respiratory disease and 30 days thereafter, were examined for serum antibody titers against the following viruses. Serum antibody titers for infectious bovine rhinotracheitis (IBR) virus, bovine respiratory syncytial virus (RSV) and bovine virus diarrhea/mucosal disease (BVD) virus were determined by neutralization tests described earlier [7, 8, 13], and those against parainfluenza-3 (PI-3) and adenovirus-7 (Ad-7) by hemagglutination inhibition tests [6, 12].

Statistical analyses: The χ^2 test was used to compare the incidence and relapse rates of repiratory disease, and the Student t-test was used for all other statistical significance determinations.
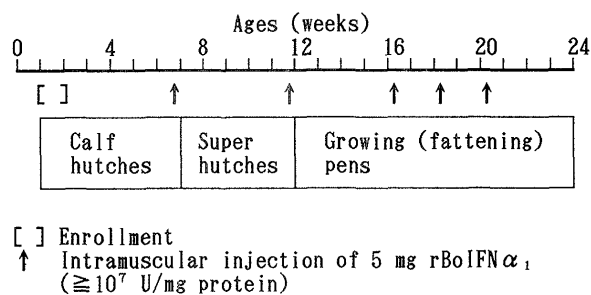
RESULTS

Effects of rBoIFNα_1 on the incidence and relapse of respiratory disease: The effects of rBoIFNα_1 on the incidence and recurrence of respiratory distress in cattle are summarized in Table 2. The rates of incidence and recurrence in rBoIFNα_1-treated cattle were 23% and 14%, respectively, whereas the values in the placebo control were 80% and 46%, respectively. The numbers of sick cattle were 24 out of 30 heads in the placebo control and 7 out of 30 heads in the treatment group. The total and mean of sick days were 154 and 6.42 days in the placebo control and 31 and 4.43 days in the treatment group, respectively, showing that rBoIFNα_1-treated cattle recovered earlier than the placebo control. The mean and standard error of the clinical scores for sick cattle were 2.34±0.36 in the placebo control and 1.92±0.35 in the treatment group. All of these parameters were significantly different at P<0.05 in both groups.

In bacteriological examinations, *P. multocida* was detected in nasal discharge samples from almost all sick cattle. Serology revealed dominant seroconversion for Ad-7, BVD and PI-3 in sick calves. However, no clear effect of rBoIFNα_1 treatment on antibody titers against these viruses was observed.

Effects of rBoIFNα_1 on mortality and average body weight gain per day: One case in 30 placebo control cattle died in the observation period of 6 months whereas none was dead in rBoIFNα_1-treated cattle (Table 3). Furthermore, the mean gain of body weight per day in 6 months in both placebo control and rBoIFNα_1-treated groups was 1.340 and 1.365, respectively, indicating better growth in rBoIFNα_1-treated calves.

Table 2. Effects of rBoIFN α_1 on the incidence of BRD in young male cattle in natural field situations

Treatment	No. of cattle	Incidence of BRD	Relapse rate	Sick days[a]		Average of clinical score
				Total	Mean	
Placebo	30	24/30 (80%)	11/24 (46%)	154 (n=24)	6.42	2.34±0.36
rBoIFNα_1	30	7/30 (23%)[b]	1/7 (14%)[b]	31[b] (n=7)	4.43[b]	1.92±0.35[b]

a) Total sick days represent the accumulated period of sick days in cattle with apparent signs of respiratory disease, and mean sick days represent the average period of sick days in a cattle affected with respiratory disease.
b) $P<0.05$, when compared to placebo controls.

Table 3. Effects of rBoIFNα^1 on mortality and average daily body weight gain in young male cattle in natural field situations

Treatment	No. of cattle	No. of deaths	Average daily body weight gain[a]
Placebo	30	1	1.340±0.070
rBoIFNα_1	30	0	1.365±0.050

a) Mean and standard error of body weight (kg/day) measured at 6 months of age.

DISCUSSION

The results of the present study are summarized as follows: The administration of rBoIFNα_1 into growing calves resulted in reduced mortality and incidence of respiratory diseases. Even rBoIFNα_1-treated calves affected by respiratory disease showed less severe clinical symptoms, shortening of sick days and less recurrence of respiratory disease. Therefore, rBoIFNα_1 appears to have a prophylactic effectiveness in controlling bovine respiratory disease.

The respiratory disease complex involving many interactions of different stresses and pathogens is one of the major health problems in the production of domestic food animals. Clinically healthy cattle can harbor both viral and bacterial pathogens. They are not often quickly overcome by the infectious agent and sickened. A variety of stresses including weaning, change of feed and water and crowding predispose them to respiratory infection and promote its spread.

The mechanisms by which rBoIFNα_1 is involved in reduced respiratory disease remain unknown. The interferons have been known to have varied biological activities including the capabilities of interfering with virus replication and of modulating immune responses. These include enhanced cytotoxic activity of natural killer cells, K cells and T cells, activation of macrophages and suppressor cells, alteration of antibody synthesis and surface antigens expression such as MHC-class II antigens and Fc receptors, and decreased delayed type hypersensitivity reaction [1, 15].

Drastically profitable effects on the incidence of respiratory disease by rBoIFNα_1 treatment under field situations may be associated with reduced infection with pathogens such as bacteria and viruses detected in almost all of the sick calves. Infection with these pathogens gives a rise to immunosuppression [2, 16]. Most of studies with bovine interferon have demonstrated that suppressed polymorphonuclear leukocyte mainly neutrophil functions including migration, chemotaxis and the production of reactive oxygen species following infection with bovine herpesvirus-1 and *P. hemolytica* return to normal rapidly by rBoIFNα_1 treatment [2]. Therefore, profitable effects of rBoIFNα_1 in this study may be substantially due to immunomodulatory activities associated with the nonspecific enhancement of leukocyte functions rather than direct antiviral activity.

Based on the findings that any apparent reverse effects of rBoIFNα_1 was not found throughout this study period, rBoIFNα_1 may be at least beneficial and potentially applicable. More detailed studies concerning effective dosages, timing of administration and characterization of the condition are required to prove the efficacy of rBoIFNα_1 in natural field practices.

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