NOTE Internal Medicine

No Effect of Bovine Interferon-τ for Control of Calf Diarrhea and Immunomodulation in Calves

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ABSTRACT: Newborn calves received a low dose of bovine interferon-τ (bolFN-τ) orally for 4 weeks and calves that had developed diarrhea received a low dose of bolFN-τ orally for 5 days. No effects of bolFN-τ were seen in the duration of the diarrhea, or in daily weight gain. Calves received a high dose of bolFN-τ subcutaneously 3 times and they were then stimulated with bovine herpesvirus type 1 vaccine. No adverse effects were observed after the administration of bolFN-τ and lymphocyte subsets from calves did not change after the stimulation. Our results suggest that bolFN-τ does not seem protecting for preventing calves from diarrhea, recovering the health of calves with diarrhea or immunomodulation, although the treatment itself is not toxic.

KEY WORDS: calf, diarrhea, interferon-τ.

Interferons (IFNs) are cytokines that have antiviral and immunomodulatory activities. IFNs consist of three families of protein molecules, called IFN-α, IFN-β and IFN-γ. IFN-α and IFN-β are type I IFN that are produced by macrophages and dendritic cells. IFN-τ is a novel type I IFN secreted by primate trophoblast cells between days 13 and 20 of pregnancy [2, 14]. IFN-τ also has antiviral and immunomodulatory activities [1, 8, 12, 16], but exhibits very low cytotoxicity unlike other types I of IFN, even at the high levels found within the uterus during early pregnancy [11, 17, 18].

In clinical application of IFNs, IFN-α is used to treat chronic viral infections and neoplastic disorders in humans, but adverse effects associated with a high dose of IFN therapy, such as a flu-like syndrome with chills, fever, headache, myalgia, anorexia, fatigue, bone marrow suppression, nausea, reversible cardiac dysfunction, and depression, are often observed [3, 6, 13]. The safety and effects of administration of IFNs have been studied little in veterinary medicine, but several clinical investigations reported that the oral use of low-dose IFN-α was an effective for transmissible gastroenteritis (TGE) or bovine respiratory disease complex (BRDC) in calves without adverse effects [4, 5]. As an antiviral and immunomodulatory cytokine, IFN-τ is expected to be a useful therapeutic agent for the prevention and treatment of infectious disease, because of its low toxicity. In this present study we examine the effects of bovine (bo) IFN-τ for controlling calf diarrhea and immunomodulation in calves.

Recombinant bolFN-τ was provided by Katakura Industries Co., Ltd. (Saitama, Japan). It was produced by means of a baculovirus expression system and its biological activity was confirmed. It had approximately 10⁸ U/ml of antiviral activity in a cytopathic effect (CPE) inhibition assay with vesicular stomatitis virus. For oral administration, bolFN-τ was diluted in phosphate-buffered saline (PBS) to a concentration of 200 U/5 ml. For subcutaneous injection, bolFN-τ was prepared to contain 6 × 10⁹– 1.4 × 10¹⁰ U bolFN-τ in 2 ml of saline containing 0.1% porcine gelatin.

For the experiment on the prevention of calf diarrhea with bolFN-τ, 29 newborn Japanese Black calves were obtained. They were raised in a single group pen and offered milk replacer by means of an automated feeding system. Fourteen calves received a low dose of bolFN-τ (200 U/head) orally once a day for 4 weeks starting at 2 days after birth. The remaining 15 calves were untreated controls. The calves were observed daily for the degree of diarrhea with the clinical scores shown in Table 1. Daily weight gains through weaning were calculated.

For the experiment in the treatment of diarrhea with bolFN-τ, 39 Japanese Black calves aged 2–30 days old that had developed diarrhea were obtained. The calves were reared with their dams and suckled naturally. Nineteen

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Table 1. Clinical scores for the assessment of the severity of diarrhea

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feces</td>
<td>Normal 0</td>
</tr>
<tr>
<td>Appetite</td>
<td>Normal 0</td>
</tr>
<tr>
<td>Behavior</td>
<td>Normal 0</td>
</tr>
<tr>
<td>Dehydration</td>
<td>None 0</td>
</tr>
</tbody>
</table>
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Table 2. Effects of a low dose of boIFN-τ on prevention of calf diarrhea

<table>
<thead>
<tr>
<th>Group of calves</th>
<th>Morbidity of diarrhea (%)</th>
<th>Duration of diarrhea (days)</th>
<th>Clinical scores</th>
<th>Daily weight gain(^a) (kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>boIFN-τ(^a) (n=14)</td>
<td>92.9</td>
<td>3.9 ± 3.0</td>
<td>6.5 ± 6.4</td>
<td>0.81 ± 0.11</td>
</tr>
<tr>
<td>control(^b) (n=15)</td>
<td>100</td>
<td>3.5 ± 2.0</td>
<td>8.7 ± 8.0</td>
<td>0.77 ± 0.12</td>
</tr>
</tbody>
</table>

a) Calves received boIFN-τ (200 U/head) orally once a day for 4 weeks starting at 2 days after birth. 
b) Calves were untreated. 
c) Daily weight gain through weaning was calculated.

daily weight gain through weaning was calculated.

calves received a low dose of boIFN-τ (200 U/head) and 20 calves received PBS as a placebo. These were orally administered daily for 5 days starting at the onset day of diarrhea and the degree of clinical diarrhea was monitored daily for 12 days. The calves were weighed at the onset day of diarrhea, and at 7 days after the final administration of boIFN-τ for the boIFN-τ treatment for immunomodulation in calves, 9 head of 2–4 month-old healthy Holstein calves were assigned to 2 treatment groups. The 5 calves in the boIFN-τ treatment group received a high dose of boIFN-τ (10^8 U/kg) subcutaneously three times every 3 days. The remaining 4 calves in the placebo group were given 2 ml saline without boIFN-τ. The day after the third injection, all of the calves were stimulated with live attenuated bovine herpesvirus type 1 (BHV-1) vaccine (Biken Laboratories, Inc, Kyoto, Japan). Blood samples were taken from calves at vaccination, on days 2, 7, 14, 21 and 28 after the vaccination. Clinical features were observed and body temperature was taken at least twice a day over the experiment period. The number of peripheral white blood cells (WBC) was monitored during the boIFN-τ treatment.

Numbers of lymphocyte subsets in peripheral blood mononuclear cells (PBMCs) of calves were analyzed as follows: PBMCs were isolated from whole blood by Ficoll-Paque (Amersham Biosciences, Sweden) gradient centrifugation. PBMCs were stained with specific monoclonal antibodies, including MM1A (anti bovine CD3, VMDR, Pullman, WA, U.S.A.), CACT138A (anti bovine CD4, VMDR, Pullman, WA, U.S.A.), CACT80C (anti bovine CD8, VMDR, Pullman, WA, U.S.A.) and GB21A (anti γ-chain of bovine γδTCR, VMDR, Pullman, WA, U.S.A.). After incubation, the cells were reacted with FITC-conjugated anti-mouse IgG-A-M (Cappel, ICN Pharmaceuticals, Inc, Ohio, U.S.A.) and analyzed on a flow cytometer (EPICS EX, Coulter Corporation, Miami, FL, U.S.A.).

For the evaluation of lymphocyte proliferative response, PBMCs were suspended at a concentration of 1 x 10^6 cells/ml in RPMI1640 medium containing 10% fetal calf serum (FCS). These 1 x 10^6 cells were stimulated with 5 μg of ConA/ml in a 96-well plate at 37°C in 5% CO2 for 72 hr. Evaluation of cell proliferation was based on the cellular conversion of a tetrazolium salt into a formazan product [10]. The stimulation index (SI) was calculated by the following formula; SI= sample’s absorbance at 540 nm/control absorbance at 540 nm.

The antibody to BHV-1 was measured by virus-neutralizing test. Briefly, serial 2-fold dilutions of serum samples were mixed with an equal volume of BHV-1 Los Angeles strain suspensions containing 200 TCID₅₀/0.1 ml of infective titer, and 0.1 ml of the mixtures were incubated in 96-well plates for 20hrs at 37°C. Madin-Darby bovine kidney (MDBK) cells were suspended in Eagle’s minimum essential medium containing 10% FCS, added to the mixtures, and incubated for 5 days at 37°C. Virus-neutralizing antibody titers were expressed as the reciprocal of the highest serum dilution that completely inhibits the CPE. The data were analyzed for statistical significance by Student’s t-test.

In the experiment on the prevention of calf diarrhea with a low dose of boIFN-τ, the morbidity and duration of diarrhea were 92.9% and 3.9 days in the calves in the group that received IFN, and 100% and 3.5 days in the calves in the control group. No significant differences between the IFN and control groups were seen in the clinical score or daily body weight gain through weaning (Table 2). No calf had anorexia after the administration of IFN-τ. In the experiment on the treatment of calf diarrhea with a low dose of boIFN-τ, the duration of diarrhea in the IFN treatment group and in the placebo group was 8.9 days and 8.8 days, respectively. No significant differences between the IFN and placebo groups were seen in the clinical score or daily weight gain through out the 12 days of the experiment (Table 3).

In the experiment on the immunomodulation in calves
with a high dose of boIFN-τ, 6 to 12 hr after the administration of boIFN-τ or placebo, calves showed transient fever that returned to normal within 24 hr. There was no difference between the calves that received IFN-τ and the placebo calves in the mean body temperature. No anorexia or leukopenia was observed during the experiment period. The number of CD3, CD4, CD8 and γδ positive T cells in PBMCs from calves in the IFN-receiving group did not change significantly after vaccination. No significant differences were seen between the IFN group and the placebo group in the number of positive cells at days 2, 7, 14, 21 and 28 (Table 4). No significant differences were seen in the SI value of lymphocyte proliferative response assay between IFN-receiving calves and placebo calves on any of the examination days (data not shown). All calves developed virus-neutralizing antibody to BHV-1 14 days after vaccination. At 28 days after vaccination, the virus-neutralizing antibody geometric mean titer (GMT) in the serum of calves in the IFN-receiving group and the placebo group was 12.0 and 9.5, respectively (not significant).

In this study, the oral administration of a low dose of boIFN-τ and the subcutaneous administration of a high dose of boIFN-τ induced no adverse effects, such as fever, leukopenia or anorexia. Previous studies reported that adverse effects associated with IFN treatment were observed in cattle injected with IFN-γ, the total number of leukocytes decreased with the rise in body temperature, and the numbers of CD8 positive cells decreased [7, 15]. It is of interest that boIFN-τ is less toxic, unlike boIFN-γ when used at a high dose in vivo.

Nevertheless, boIFN-τ was not effective on diarrhea or immunomodulation in calves despite having biological activity in vitro. The effects of boIFN-τ in vivo demonstrated that intramuscular administration of boIFN-τ caused a rise in body temperature and a decrease in plasma progesterone in dairy cows [9], but the activities of boIFN-τ in calves were unknown. The age and immune status of the animals might affect their sensitivity to IFN. In our present study, boIFN-τ may not have induced the immune response of the calves due to the immaturity of the immune system of the calves.

Diarrhea in calves can be caused by many different agents, and involve many interactions with the immune systems of calves and stress factors associated with environment, nutrition, and management. Therefore, it might be difficult to demonstrate the effects of boIFN-τ for calf diarrhea in a field condition. Several studies have shown that IFN-α seemed to have the property of enhancing weight gain in calves and piglets [4, 5]. This weight gain effect might be a secondary effect of IFN resulting in a generalized elevation of immunocompetence and increased general health of the host.

Furthermore, the stability of boIFN-τ must be considered. The boIFN-τ might be very sensitive to heat and dilution, or might have lost activity in vivo immediately after administration. For clinical use of boIFN-τ, further studies are expected to determine the effective dosage, route and timing of the administration of boIFN-τ for calves in a field situation.

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