Effect of Low-Dose Human Interferon-alpha on Shipping Fever of Thoroughbred Racehorses

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To assess the effect of human interferon-alpha (IFNα) on shipping fever of Thoroughbred racehorses subjected to long-distance transportation, an IFNα preparation was orally administered to 48 horses three times (once daily, 3 successive days) before transportation (IFNα group). In the control group (25 horses), maltose was administered in the same way. These treatments induced no abnormal findings in Thoroughbred racehorses before transportation. Immediately after transportation, significant increases in rectal temperature were observed in both treatment groups, whereas the rectal temperature of the IFNα group tended to be lower than that of the control group. Although WBC, Fbg, and SAA immediately after transportation were significantly increased due to transportation in both groups, the extent of the increases in the IFNα group was significantly smaller than in the control group. Long-distance transportation had a relatively profound impact on Thoroughbred racehorses, which was mitigated by IFNα treatment.
Key words: human interferon-alpha, low-dose, oral administration, thoroughbred racehorses, shipping fever

Introduction

In long-distance transportation of Thoroughbred racehorses there have been issues with pyrexia (shipping fever) or with transport pneumonia, which occurs with the aggravation of shipping fever. These problems have been caused mainly by transportation stress and/or degradation of the environments in trucks for transportation of horses [10, 11]. Although these diseases have shown a tendency to decrease in incidence because of improvements in transport environments and in the management of individual Thoroughbred racehorses before transportation, no decisive methods for their prevention have yet been established, and they are still among the major risks posed by equine long-distance transportation [4, 11].

Human interferon-alpha (IFNα) is a protein produced in the body mainly during virus infection; it has known immunostimulatory and anti-viral activity [12]. High-dose injections of IFNα have been given to treat tumors and viral infections in the medical care of humans and small animals [3, 7, 8, 13]. However, similar immunostimulation activity has been reported from the oral administration of low-dose human IFNα [1, 2, 12, 13]. Although the mechanisms of action have not been completely elucidated, the binding of IFNα to the receptors of the immune-related cells commonly present in the pharynx and esophagus may trigger the cytokine network to promote the activation of immune cells [1]. The oral administration of low-dose human IFNα to horses has been reported as effective in inflammatory airway diseases and in the prevention of shipping fever in young racing Thoroughbreds [5, 9]. However, there have been no reports on the oral

administration of human IFNα for preventing shipping fever in Thoroughbred racehorses which have trained with a sufficient load for racing.

We investigated the efficacy of low-dose human IFNα in shipping fever of Thoroughbred racehorses locked and loaded for racing. The drug was given orally before long-distance transportation of the horses for participation in racing.

Materials and Methods

Drug administered

The IFNα (IFNα: 200 IU/g; BIMURON®, BioVet, Tokyo, Japan) used was human native IFNα produced for use in powder form for animals by the culture of human cells, using maltose as a base.

Transportation

We used trucks exclusively designed for transportation of horses, which have a carrying capacity of six horses in the direction of travel. An equipped air-conditioning system was used as the need arose. If air-conditioning was unnecessary, the truck could be naturally ventilated by drawing fresh air in through the window. The subjects were 73 Thoroughbred racehorses (52 males or geldings, 21 females; mean ± standard deviation (SD); age, 3.6 ± 1.3 years old) transported from the Ritto Training Center of the Japan Racing Association (JRA Ritto) to the Hakodate Racecourse of JRA (JRA Hakodate) or the Sapporo Racecourse of JRA (JRA Sapporo). The duration of transportation was approximately 20 hr from JRA Ritto to JRA Hakodate and approximately 26 hr from JRA Ritto to JRA Sapporo. The period of the experiment was 4 months (May to August).

The 48 horses randomly sampled from among the 73 horses investigated were orally administrated the IFNα (1.25 g/head/day: IFNα group); while the remaining 25 horses were orally administered maltose as the drug base (Maltose, Wako pure chemicalindustries, Osaka, Japan; 1.25 g/head/day: control group). On the basis of the results of a previous study, the drugs were administered once daily and continued for 3 successive days before transportation (including on the day of transportation) [5]. Rectal temperatures (RT) were measured and blood sampled immediately before the initial administration of IFNα or maltose, as well as just before transportation and immediately after transportation.

Blood examination

Blood samples were collected from the jugular veins of the animals in plain blood collection tubes (VP-P100K, Terumo, Tokyo, Japan) or tubes containing sodium citrate buffer (VP-C050, Terumo) or EDTA (VP-DK050K, Terumo). Blood collected with the tube containing sodium citrate buffer was centrifuged (4°C, 2,000 g, 10 min) to obtain plasma and measure plasma fibrinogen (Fbg) concentrations by the salting-out method (Iatroset Fbg, Mitsubishi Kagaku Iatron, Tokyo, Japan), using human Fbg as a standard. For blood collected with the EDTA tube, white blood cell counts (WBC) in peripheral blood were measured by an automated hemocytometer (K-4500, Sysmex, Hyogo, Japan). Blood collected with the plain tube was centrifuged (25°C, 2,000 g, 10 min) to obtain serum to measure serum amyloid A (SAA) concentrations by latex agglutination turbidimetric immunoassay (LZ test "Eiken" SAA, Eiken chemical, Tokyo, Japan), using equine SAA as a standard [6].

Statistical analysis

The data were analyzed by analysis of variance for comparison within each group or by Mann Whitney's U test for intergroup comparison, with P<0.05 considered significant. The results are shown as mean ± standard deviation. In addition, the proportions of animals that demonstrated clinically evident abnormalities in RT, WBC, Fbg, and SAA ($\geq$39.0°C, $\geq$15,000/mm^3, $\geq$400 mg/dl, and $\geq$1 μg/ml, respectively) were calculated [5, 6]. The horses' general condition (physical condition, appetite, and feces) was also observed.

Results

In both treatment groups, no statistically significant differences in RT, WBC, Fbg, or SAA were observed between the measurements taken just before the initial administration of IFNα or maltose and those taken immediately before transportation (Table 1). In addition, no abnormalities were seen in the horses' general condition.

In both groups, RT immediately after transportation was significantly higher (P<0.05) than immediately before transportation (Table 1). RT immediately after transportation in the IFNα group tended to be lower than in the control group (Table 1). Furthermore,

Table 1. Changes of rectal temperature, fibrinogen, and serum amyloid A in just before the initial administration of IFNα, immediately before transportation, and immediately after transportation

Group	Sampling	Rectal temperature (°C)	WBC (/mm³)	Fbg Fbg (mg/dl)	SAA SAA (μg/ml)
Control	pre-administration*	37.9 ± 0.1	9,460 ± 2,137	237.5 ± 42.7	0.0 ± 0.1
	pre-transportation**	37.9 ± 0.1	8,608 ± 1,363	230.4 ± 33.1	0.0 ± 0.1
	post-transportation***	38.5 ± 0.6^{A,B}	11,708 ± 3,134^{A,B,a}	304.2 ± 76.4^{A,B,b}	6.5 ± 15.4^{C,D,c}
IFNα	pre-administration	37.9 ± 0.1	9,535 ± 1,962	235.2 ± 27.2	0.0 ± 0.1
	pre-transportation	37.9 ± 0.1	8,373 ± 1,419	238.6 ± 30.6	0.0 ± 0.1
	post-transportation	38.3 ± 0.5^{A,B}	10,344 ± 3,000^{A,B,a}	262.0 ± 42.4^{A,B,b}	1.0 ± 2.9^{C,D,c}

*pre-administration: just before the initial administration of IFNα. **pre-transportation: immediately before transportation. ***post-transportation: immediately after transportation. The data were analyzed by analysis of variance for comparison within each group or by Mann Whitney's U test for intergroup comparison, value of P<0.05 were considered significant. ^{A} Significantly different from the pre-administration value at each sampling time (P<0.01). ^{B} Significantly different from the pre-transportation value at each sampling time (P<0.01). ^{C} Significantly different from the pre-administration value at each sampling time (P<0.05). ^{D} Significantly different from the pre-transportation value at each sampling time (P<0.05). ^{a,b,c} Significant differences between the same superscripts (P<0.05).

Table 2. The percentage of horses showing abnormal values just before the initial administration of IFNα, immediately before transportation, and immediately after transportation

Group	Sampling	Rectal temperature (%)	WBC (%)	Fbg (%)	SAA (%)
Control	pre-administration*	0	0	0	0
	pre-transportation**	0	0	0	0
	post-transportation***	16.0	20.0^a	12.0^b	44.0^c
IFNα	pre-administration	0	0	0	0
	pre-transportation	0	0	0	0
	post-transportation	14.6	8.3^a	0.0^b	16.7^c

Rectal temperature: Percentage of horses showing rectal temperature of ≥39.0. WBC: Percentage of horses showing WBC value of ≥15,000/mm³. Fbg: Percentage of horses showing Fbg value of ≥400 mg/dl. SAA: Percentage of horses showing SAA value of ≥1.0 μg/ml. The data were analyzed by Mann Whitney's U test for intergroup comparison, with values of P<0.05 considered significant. *,**,***,a,b,c: See Table 1 for key.

14.6% of the animals in the IFNα group and 16.0% in the control group had RT of 39.0°C or higher, although there was no significant difference between the two groups (Table 2). No further increases in rectal temperature and deterioration in general condition were found 2 days after transportation, and all the horses that developed fevers improved within a few days.

In both treatment groups WBC immediately after transportation was significantly greater than immediately before transportation (P<0.05). However, WBC just after transportation in the IFNα group was significantly lower (P<0.05) than in the control group (Table 1); moreover, 8.3% of horses in the IFNα group and 20.0% in the control group had WBC of 15,000/mm³ or higher immediately after transportation (Table 2).

Fibrinogen levels immediately after transportation were significantly higher (P<0.05) than immediately before transportation in both groups, and Fbg just after transportation in the IFNα group was significantly lower (P<0.05) than in the control group (Table 1). Just after transportation, 12.0% of animals in the control group had Fbg levels greater or equal to 400 mg/dl, whereas no animals in the IFNα group had such high Fbg levels; this difference was significant (P<0.05) (Table 2).

Significant increases (P<0.05) in SAA levels just after transportation compared with those immediately before transportation were observed in both groups. The SAA level in the IFNα group was lower (P<0.05) than in the control group. The proportion of horses in

the control group with abnormal SAA just after transportation (44.0%) was higher (P<0.05) than in the IFNα group (16.7%) (Table 2).

Discussion

Low-dose oral administration of IFNα, a technique that has recently been used in clinical settings, has been reported to show efficacy in the treatment of inflammatory respiratory diseases and the prevention of shipping fever in young Thoroughbred racehorses during long-distance transportation [5, 9]. Hobo *et al.* reported that oral administration of human IFNα at 0.5 IU/kg/day resulted in preferable outcomes in respect of prevention of shipping fever in young Thoroughbred racehorses (2 years old) [5]. They also stated that multiple administrations of human IFNα reduced the incidence of shipping fever, and continuous human IFNα for 3 days was more effective than a single administration for preventing the disease [5]. In this study, referring to the reports by Hobo *et al.* [5], we decided on duration of administration of low-dose human IFNα of 3 days before transportation, including the day of transportation. On the basis of the average body weight of a racing Thoroughbred (450 to 500 kg) we chose a drug dose rate of 1.25 g/day/head (250 IU/day/head), without variation. Determination of dosage on the basis of individual body weights would have been difficult in a clinical situation.

The safety of low-dose human IFNα has already been confirmed in previous reports on young Thoroughbred racehorses [5], and we observed no hematological or clinical condition abnormalities in during or after administration of the drug. We confirmed that low-dose human IFNα could be safely used in Thoroughbreds trained with a sufficient load for racing.

Immediately after transportation, RT, WBC, and inflammatory markers (Fbg and SAA) were significantly higher than immediately before transportation. These results indicate that long-distance transportation yields considerable stress on the horses, and that the stress itself could not be completely mitigated by the administration of low-dose human IFNα. However, comparison between the treatment groups revealed that the extent of the increase in RT in the IFNα group just after transportation tended to be smaller than in the control group.

As for the IFNα group, increases of WBC, Fbg and

SAA were inhibited significantly in comparison with the control group, but shipping fever was not prevented. Investigation of the dosage and number of doses was insufficient in this study. Accordingly we think that detailed consideration of these factors is required to clarify the effect of treatment regimen IFNα administration to Thoroughbred racehorses.

In this study, Fbg and SAA values in the IFNα group were significantly lower than in the control group. We think that this was due to IFNα administration inhibiting the intravital inflammatory degree due to transportation. We also think that the other acute phase proteins it will be necessary to measure in the future.

In this study, we did not examine the mechanism of IFNα action, and it will be necessary to clarify this mechanism in the future.

In conclusion, long-distance transportation had a relatively profound impact on Thoroughbred racehorses, which was mitigated by IFNα treatment.

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