FULL PAPER *Internal Medicine*

Effects of pre-shipping marbofloxacin administration on fever and blood properties in healthy Thoroughbreds transported a long distance

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ABSTRACT. The present study evaluated the effects of single-dose marbofloxacin in protecting horses against fever associated with transportation using 48 healthy Thoroughbreds. All horses were premedicated with interferon-α (0.5 U/kg, sublingually, every 24 hr) for 2 days before transportation and on the day of transportation. Horses were randomly assigned to receive marbofloxacin (2 mg/kg, IV, once; MRFX group), enrofloxacin (5 mg/kg, IV, once; ERFX group) or saline (0.9% NaCl) solution (10 m*l*, IV, once; control group) ≤1 hr before being transportation. Each group contained 16 horses (8 males, 8 females). Horses were transported 1,210 km using commercial vans over the course of approximately 26 hr. Clinical examinations and hematologic analyses were performed on all horses both before and after transportation. Post-transportation neutrophil to lymphocyte ratios were significantly lower in horses in the MRFX group compared with the control horses. The serum amyloid A levels were significantly lower in horses in the MRFX group and ERFX group compared with the control horses. Regarding the post-transportation rectal temperatures, fever was detected in 0 horses and 1 horse in the MRFX and ERFX groups, respectively, whereas fevers exceeding 39.1°C were detected in 2 horses in the control group. Additionally, the number of essential post-transportation treatments provided by veterinarians was reduced 3-fold in the MRFX and ERFX groups compared with the saline group. MRFX provided ERFX-like protection against fever associated with long-distance transportation, yielding significantly better protection than saline. Administration of MRFX just before transportation deserves a further study for efficacy in preventing horse fever associated with transportation.

KEY WORDS: equine, marbofloxacin, prevention, transportation-associated fever

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With the globalization of horse racing, long-distance transportation of racehorses via airplane or horse trailer has become common. Fever associated with transportation is a serious symptom that can disrupt training and racing schedules, and pneumonia and pleuropneumonia due to bacterial infection resulting from long-distance transportation potentially increase equine mortality [14, 15]. Prevention of this symptom improves the planning of training and racing schedules, potentially minimizing the physical deconditioning associated with such transportation.

Research into fever associated with transportation has been performed from various perspectives [13–16, 19]. Fever associated with transportation is thought to be induced primarily by transportation stress and deterioration of the environment in the truck, and it is typically observed 20 hr or more after the start of transportation [13, 15, 16]. During long-distance transportation, the horse's elevated head position induces inflammation, obstruction of tracheal mucociliary clearance and changes in host immunity, with associated increases in bacterial abundance in the lower respiratory tract [18]. The horse's bronchoalveolar region can become infected by opportunistic pathogens, including *Streptococcus equi* subsp. *zooepidemicus* (*S. zooepidemicus*), a resident of the tonsillar tissues and trachea that is considered the main causative organism of shipping fever [14–16, 22].

Some of these previous studies have suggested prophylactic treatments intended to prevent fever associated with transportation. A decrease in the incidence of fever associated with transportation and improvement in clinical condition was reported for horses orally administered interferon-α (IFN-α) for 3 consecutive days before transportation to activate the immune system [1]. However, this protocol did not completely prevent fever associated with transportation, so further prophylactic measures are needed. Based on these previous papers, we think that activation of immunity by IFN-α administration before transportation, together with the presence in the bronchoalveolar region during transportation of an antimicrobial agent that is effective against the bacteria that exist in the tonsillar tissues and trachea, is important for effective prevention of fever associated with transportation. However, most of the antimicrobial agents used in horse clinics have short active durations [17], and it is difficult to maintain an effective concentration in the body

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for long periods after a single dose before transportation.

In contrast, new broad-spectrum quinolone antibiotics, including enrofloxacin (ERFX) and marbofloxacin (MRFX), have been used clinically as long-acting antimicrobial agents, including for treatment of infections of the respiratory system [2, 6–9]. Following intravenous administration in adult horses, these antibiotics are transported effectively to the bronchoalveolar region, and blood concentrations remain high throughout the 24 hr after dosing [6, 7, 10]. In a long-distance-transport model, Davis *et al.* examined the efficacy of ERFX administration after transportation for the purpose of treatment [8]. Separately, we reported that fever associated with transportation was significantly decreased by prophylactic administration of 5 mg/kg ERFX [20]. However, the ERFX formulation is strongly alkaline and highly tissue invasive, potentially resulting in necrosis of tissue from leaking injections; the risk of leakage is increased, because of the large dose volume required. In contrast, MRFX exhibits little local tissue damage, even when administered by the subcutaneous or intramuscular route [6, 7]. However, there are, to our knowledge, no previous reports of the administration of MRFX for the prevention of fever associated with transportation.

We therefore investigated the efficacy of MRFX in protecting against fever associated with transportation. ERFX was used as a comparative treatment, and the study subjects (Thoroughbred horses) also received sublingual IFN-α for 3 days before transportation, including the transportation day.

MATERIALS AND METHODS

Animals: The experiment was approved by the Animal Care and Use Committee at the Hidaka Training and Research Center of the JRA.

Forty-eight clinically healthy Thoroughbred horses (24 males, 24 females; age, 2 years old) were used for the study. The animals had been purchased for sale in the following year's trained horse sale and so had received training for approximately 6 months at the Hidaka Training and Research Center of the JRA in Hokkaido Prefecture (JRA Hidaka). The animals were destined for the trained horse sale held at the JRA's Nakayama Racecourse in Chiba Prefecture (JRA Nakayama). Prior to transport, we confirmed that the horses were not suffering from severe respiratory diseases, such as pneumonia or pleuropneumonia.

All animals received IFN-α (BIMURON, Biovet, Tokyo, Japan) sublingually at 0.5 U/kg, every 24 hr, for 3 consecutive days before transportation, including the day of transportation [1]. The horses then were randomly divided into 3 groups of 16 horses (8 males, 8 females) each: an MRFX administration group (MRFX group), an ERFX administration group (ERFX group) and a control group. The horses in the 3 groups were administered, by intravenous injection, MRFX (Marbocyl 10%, Meiji, Tokyo, Japan) at 2 mg/kg, ERFX (Baytril 5%, Bayer, Osaka, Japan) at 5 mg/kg or saline (Otsuka Saline Injection, Otsuka, Tokyo, Japan) at 10 m*l*, respectively. These doses were determined based on previous studies [6, 7, 20]. Intravenous injections were administered no more than 1 hr before the animals were loaded onto a van. The horses were transported for approximately 26 hr, and we performed a clinical examination and hematologic analysis before and after transportation. MRFX and ERFX are used in general equine practice abroad [2–8, 10, 11, 21], but use of them is limited by the Ministry of Agriculture, Forestry and Fisheries in Japan.

Transportation: The departure point was JRA Hidaka, and the destination was JRA Nakayama. The duration of transportation was approximately 26 hr, and the distance was 1,210 km. The land portion of the route was 1,100 km (expressway 1,000 km and national highway 100 km), and the sea portion of the route was 110 km. We used a large ferry for the sea portion of the route (from Hakodate Port in Hokkaido Prefecture to Aomori Port in Aomori Prefecture); the ferry trip took 3 hr and 30 min.

We used 10 commercial vans, each of which could be loaded with 6 horses and was designed exclusively for horse transportation. The structure of the vans was the same for all 10. The horses were fed twice during transportation (at comfort stops 7 hr and 23 hr after the start of transportation); they were individually fed assorted types of feed at 1 kg/head. Water was always in free supply. Travel breaks were taken for about 15 to 30 min every 4 hr, and the insides of the trucks were ventilated at every break. After transportation, all horses were subjected to training for 7 days.

Clinical examination and hematologic analysis: Before, during and after transportation, the horses were also examined by ocular inspection and palpation for the presence of any locomotive or digestive system signs associated with the medication (MRFX, ERFX or saline). Rectal temperature was taken with a mercury thermometer just before and just after transportation and at one day after arrival. Horses with an elevated rectal temperature after transportation were treated with an antimicrobial agent based on the judgment of veterinarians with abundant experience in horse transportation; thier judgments were based on evaluation of the degree of rectal temperature elevation and the clinical manifestations of the horses. In accordance with the clinical signs, horses were given a penicillin–streptomycin combination (Mycillin, Meiji) at penicillin 8,000 U/kg and streptomycin 10 mg/kg, IM, every 24 hr, or cephalothin sodium (Coaxin, Chemix, Yokohama, Japan) at 20 mg/kg, IV, every 8 hr, to treat fever associated with transportation. The penicillin–streptomycin combination and cephalothin sodium were selected in accordance with the susceptibility of the bacteria typically isolated from lower respiratory infections in horses [14, 19]. In addition, a penicillin–streptomycin combination is authorized and used at this dose routinely and widely in Japan. Horses with temperatures above 38.5°C were administered one of these 2 antimicrobial agents as follows: horses that lacked systemic or respiratory symptoms received the penicillin-streptomycin combination; animals exhibiting depression, respiratory symptoms such as coughs or signs by ocular inspection received cephalothin sodium.

Blood samples were collected from the jugular veins of the animals in plain blood collection tubes (VP-P100K,

Table 1. Rectal temperatures and blood parameters before and after transportation in horses dosed prophylactically with IFN-α and quinolone antibiotic

Group	Sampling (elapsed time)	Rectal temperature (°C)	Peripheral blood				
			WBC (/mm^3)	N/L ratio	SAA (μg/m*l*)	PCV (%)	Hgb (g/d*l*)
Control	Before transportation (0 hr)	38.0 ± 0.4	9,681 ± 1,466	1.4 ± 0.5	0.8 ± 0.1	38.0 ± 4.1	14.5 ± 1.4
	After transportation (24 hr)	38.5 ± 0.5	10,613 ± 2,646	2.5 ± 1.1	153.8 ± 319.1	47.3 ± 4.5	15.3 ± 1.3
	Next day after transportation (48 hr)	38.3 ± 0.3	11,475 ± 2,197	2.0 ± 1.9	160.0.±348.5	45.2 ± 5.6	14.8 ± 1.5
MRFX	Before transportation (0 hr)	38.0 ± 0.2	9,181 ± 2,088	1.4 ± 0.5	0.8 ± 0.2	38.8 ± 3.1	14.8 ± 1.2
	After transportation (24 hr)	38.3 ± 0.2	9,325 ± 1,725	1.7 ± 0.6*	7.1 ± 19.8*	49.6 ± 5.3	16.0 ± 1.4
	Next day after transportation (48 hr)	38.3 ± 0.3	10,719 ± 1,412	1.3 ± 1.0	9.1 ± 29.0*	48.3 ± 4.6	15.9 ± 1.3
ERFX	Before transportation (0 hr)	37.9 ± 0.2	9,006 ± 1,225	No Data	0.8 ± 0.1	39.8 ± 3.5	15.0 ± 1.3
	After transportation (24 hr)	38.3 ± 0.3	8,894 ± 1,748	No Data	7.0 ± 23.9*	50.4 ± 2.1	16.2 ± 0.7
	Next day after transportation (48 hr)	38.3 ± 0.3	10,081 ± 1,460	No Data	12.5 ± 43.7*	46.7 ± 5.9	15.3 ± 1.8

Data are expressed as the mean ± SD. *Significant difference as compared with the control group (P<0.05).

Terumo, Tokyo, Japan) or tubes containing EDTA (VC-C50, Terumo). The EDTA blood was used to measure the peripheral white blood cell (WBC) count and hemoglobin (Hgb) concentration by automatic hemocytometer (K-4500, Sysmex, Kobe, Japan). The EDTA blood also was used to measure packed cell volume (PCV) with a hematocrit capillary tube and to calculate the neutrophil to lymphocyte ratio (N/L ratio) after preparation of smears by a fixation method (Diff-Quick 16920, Sysmex). For horses in the MRFX and control groups, two hundred cells per representative area were counted, and the N/L ratio was calculated.

Sera were isolated from the samples collected in plain blood collection tubes following clotting and centrifugation (2,000 × *g*, 10 min, 25°C). The serum amyloid A (SAA) concentration was measured by the latex agglomeration method (LZ test "Eiken" SAA; Eiken Chemical, Tokyo, Japan) using equine standard sera with SAA concentrations ranging from 0.0 to 400.0 μg/m*l*; these standards were produced by methods described in previous reports [12].

Statistical analysis: Data are expressed as the mean ± SD. For the N/L ratios, a Mann-Whitney U-test was used to compare the control and MRFX groups. For other values, variances were evaluated via the Bartlett test. Homogeneous data were subjected to the one-factor ANOVA. Nonhomogeneous data were subjected to a nonparametric test (Kruskal-Wallis). All analyses were upper tailed. Values of P<0.05 were considered significant.

RESULTS

No side effects, including locomotive or digestive system signs, were associated with the medications (MRFX, ERFX or saline) before, during or after transportation.

No significant differences were found between the 3 groups in the various measurement values before transportation (Table 1).

After transportation, rectal temperatures did not differ significantly among the 3 groups: MRFX, 38.3 ± 0.2°C; ERFX, 38.3 ± 0.3°C; control, 38.5 ± 0.5°C (Table 1).

The penicillin–streptomycin combination was administered to 2 febrile horses in the MRFX group, 2 febrile horses in the ERFX group and 3 febrile horses in the control group. Cephalothin sodium was administered to 3 febrile horses in the control group (Table 2). The rectal temperatures recovered to close to normal after a single dose of the penicillin–streptomycin combination. In contrast, the 3 cephalothin sodium-treated horses in the control group required 6 ± 1 doses of cephalothin before normal temperatures were recovered.

After transportation, the N/L ratio was significantly

Table 2. Numbers of febrile horses distributed according to rectal temperature during and after transportation

Group	≤38.4°C	≥38.5°C	≥39.1°C
Control	10	4 (3A, 1B)	2 (2B)
MRFX	12	4 (2A)	0
ERFX	13	2 (1A)	1 (1A)

A: Number of horses administered penicillin-streptomycin combination.
B: Number of horses administered cephalothin sodium.

($P<0.05$) reduced in the MRFX group compared with that in the control group. The WBC counts in the peripheral blood appeared to be reduced in the MRFX and ERFX groups, but the differences were not significant (Table 1).

Both immediately after transportation and on the following day, SAA was significantly ($P<0.05$) reduced in the MRFX and ERFX groups (Table1). In addition, none of the numerical values exhibited a sex difference.

DISCUSSION

Administration of ERFX at high doses in horses has the potential to disrupt proteoglycan synthesis in articular cartilage [3, 21]. MRFX, another member of the same class of new quinolone antibiotics, may also have adverse effects on articular cartilage in immature animals. However, the reports on the toxicity of the new quinolone antibiotics were obtained from *in vitro* studies that used high doses of these agents, and there have been, to our knowledge, no reports of side effects at the usual dose range *in vivo* [4, 5, 11]. In the present study, no side effects, including signs for the locomotive and digestive systems, were observed before, during or after transportation in animals administered MRFX. Therefore, we surmise that MRFX is safe for intravenous administration (at 2 mg/kg) just before transportation in young racehorses.

After transportation, the N/L ratios and SAA concentrations in the peripheral blood were significantly lower in the MRFX group than in the control group. Also, SAA was significantly lower in the ERFX group than in the control group. In the ERFX group, we did not calculate the N/L ratio, because we confirmed a significant difference between the ERFX group and the control group in our previous study [20]. In the MRFX and ERFX groups, the number of febrile horses was lower than in the control group, and the clinical signs were slight; the rectal temperatures of the febrile horses in the MRFX and ERFX groups recovered to essentially normal values following a single dose of the penicillin–streptomycin combination.

In a previous study, it was thought that transport adversely affected the normally effective mucosal defence mechanism (e.g., ciliary motility) in the airways, leading to invasion by *S. zooepidemicus* (a common commensal microorganism in the equine tonsil and nasopharynx) into the lower airways, thus inducing acute lower airway inflammation in the affected horses [16]. The present study suggests that administration of MRFX just before transportation markedly reduces the number of *S. zooepidemicus* in and of itself and that as a result, it relieves the invasion by *S. zooepidemicus*.

Once horses have a transportation-associated fever, treatment for several days is required [14]. Although we transported the horses so that they could be used for horse racing, febrile horses cannot run. This situation results in large economic losses. In a previous study in which enrofloxacin was administered just after transportation, the results showed that the duration of treatment was several days [8]. Therefore, administration of marbofloxacin just before transportation is useful as a prophylactic measure.

A previous study tested the efficacy of IFN-α administration before transportation of racehorses [1]. That study showed that although IFN-α did not decrease the incidence of fever associated with transportation, IFN-α significantly reduced inflammation and improved the clinical conditions of horses compared with those of control horses [1]. In the present study, we prophylactically treated all the test horses with IFN-α at a dose similar to that used in the previous report [1], but we found significant differences in the numbers of horses with fever and in the results of the hematologic analysis after transportation. These findings clearly indicate that administration of MRFX or ERFX just before transportation along with IFN-α can prevent fever associated with transportation more effectively than IFN-α alone.

In our study, administration of MRFX (2 mg/kg) just before transportation was significantly more effective than saline in protecting against fever associated with transportation; the efficacy was similar to that seen with ERFX at 5 mg/kg [20]. Furthermore, because of the low tissue invasiveness, MRFX seemed more useful than ERFX. However, the administration of new quinolone antibiotics, such as MRFX, raises the concern of the potential emergence of resistant bacteria, and the Japanese Ministry of Agriculture, Forestry and Fisheries restricts its use. We selected new quinolone antibiotics, because *S. zooepidemicus* is sensitive to them and their antibacterial activity lasts 24 hr or more, but their use should be limited. The route of administration should be intravenous to minimize the adverse effect on enteral bacteria. Furthermore, we have created a new guideline for use within our racehorse medical office (JRA). Specifically, MRFX is to be used only at transportation and only for long periods of transportation (projected to exceed 20 hr); furthermore, it is to be used for animals that have previously developed shipping fever or animals with an elevated risk of developing shipping fever (e.g., horses with a history of laryngoplasty or pneumonia). Additionally, antimicrobial susceptibility tests for the post-transportation flora from tracheobronchial aspirate and feces will be performed regularly in the future to monitor for the emergence of resistant bacteria. Also, the antibiotics should ultimately be used after having taken all other steps for fever prophylaxis including ventilation of the vehicle.

In conclusion, it was thought that administration of MRFX (along with IFN-α) just before transportation in healthy Thoroughbreds is worth studying further for efficacy in preventing horse fever associated with transportation. This regimen has advantages over the similar use of ERFX, but will require a sufficient level of caution to preclude the potential development of antimicrobial resistance. Also,

studies concerning preventive administration of agents such as agents that activate immunity other than antibiotics are expected.

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