Effects of a single dose of enrofloxacin on body temperature and tracheobronchial neutrophil count in healthy Thoroughbreds premedicated with interferon-α and undergoing long-distance transportation

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Objective—To evaluate effects of a single dose of enrofloxacin (5 mg/kg, IV) on body temperature and tracheobronchial neutrophil count in healthy Thoroughbreds premedicated with interferon-α and undergoing long-distance transportation.

Animals—32 healthy Thoroughbreds.

Procedures—All horses received interferon-α (0.5 U/kg, sublingually, q 24 h) as an immunologic stimulant for 2 days before transportation and on the day of transportation. Horses were randomly assigned to receive enrofloxacin (5 mg/kg, IV, once; enrofloxacin group) or saline (0.9% NaCl) solution (50 mL, IV, once; control group) ≤ 1 hour before being transported 1,210 km via commercial vans (duration, approx 26 hours). Before and after transportation, clinical examination, measurement of temperature per rectum, and hematologic analysis were performed for all horses; a tracheobronchial aspirate was collected for neutrophil quantification in 12 horses (6/group). Horses received antimicrobial treatment after transportation if deemed necessary by the attending clinician.

Results—No adverse effects were associated with treatment. After transportation, WBC count and serum amyloid A concentration in peripheral blood samples and neutrophil counts in tracheobronchial aspirates were significantly lower in horses of the enrofloxacin group than in untreated control horses. Fever (rectal temperature, ≥ 38.5°C) after transportation was detected in 3 of 16 enrofloxacin group horses and 9 of 16 control horses; additional antimicrobial treatment was required in 2 horses in the enrofloxacin group and 7 horses in the control group.

Conclusions and Clinical Relevance—In horses premedicated with interferon-α, enrofloxacin appeared to provide better protection against fever and lower respiratory tract inflammation than did saline solution. (*Am J Vet Res* 2012;73:968–972)

Long-distance transportation of racehorses via horse trailer has become commonplace with the globalization of horse racing. Fever associated with transportation is a clinical sign that can disrupt training and racing schedules and is part of a syndrome (sometimes colloquially referred to as shipping fever in horses) that includes respiratory disease (eg, pneumonia or pleuropneumonia) caused by bacterial infection after long-distance transportation and can potentially increase mortality rate.[1–3] Prevention of transportation-associated fever would potentially simplify planning of training and racing schedules and minimize the physical deconditioning associated with transportation. Various aspects of transportation-associated fever and respiratory disease have been investigated in earlier studies.[1–5]

Abbreviations

N:L	Neutrophil-to-lymphocyte ratio
SAA	Serum amyloid A
TBA	Tracheobronchial aspirate

Transportation-associated fever is influenced by transportation stress and deterioration of the environment inside the vehicle, and it typically develops ≥ 20 hours after the start of transportation.[1–3] Bacterial infection develops in the bronchoalveolar regions of affected horses; the causative organism is usually *Streptococcus equi* subsp *zooepidemicus*, an opportunistic bacterium that is resident in the tonsillar tissues and trachea in healthy horses.[2–4,6] After the start of transportation, infection of the lower respiratory tract with opportunistic bacteria such as *S equi* subsp *zooepidemicus* is

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considered to the primary cause of transportation-associated fever.[2,6] During long-distance transportation, the horse's raised head position induces inflammation and increased bacterial abundance in the lower respiratory tract because of obstructed tracheal mucociliary clearance, and host immunity is greatly changed.[7,8] Numbers of peripheral blood leukocytes, including neutrophils, increase, and the rate of phagocytosis by peripheral blood leukocytes, are substantially reduced.[8] Circulating concentrations of SAA, a nonspecific marker of inflammation, can reflect the degree of the inflammation in horses with respiratory disease.[9]

On the basis of findings in previous studies,[1–8] attempts have been made to prevent transportation-associated fever. Oral administration of interferon-α for 3 consecutive days (to stimulate the immune system) before transportation was associated with a decrease in the severity of transportation-associated fever and improvement in clinical condition after transportation.[10,11] However, the protocol did not completely prevent fever in those horses, and further prophylactic measures are needed.

Results of the previous studies[10,11] suggest that activation of immunity by interferon-α administration before transportation, together with suitable bronchoalveolar concentrations of an antimicrobial agent that is effective against the bacteria that exist in the tonsillar tissues and trachea, could potentially provide an effective means of preventing transportation-associated fever. However, most of the antimicrobial agents used in equine clinics have short terminal half-lives,[12,13] and it is difficult to maintain effective circulating concentrations during long transportation periods after a single dose is administered. In contrast, enrofloxacin has been used clinically as a long-acting antimicrobial agent for infectious diseases, including bacterial pneumonia,[14,15] and its efficacy has been acknowledged. Enrofloxacin is a broad-spectrum fluoroquinolone antimicrobial. When administered IV at a dose of 5 mg/kg every 24 hours to an adult horse, it is transported effectively to the bronchoalveolar region (unpublished data), and serum concentrations remain high 24 hours after administration.[16] In an experimental study[14] of horses with respiratory disease following long-distance transportation, the efficacy of enrofloxacin administration at various doses was investigated for the purpose of treatment. However, to our knowledge, no reports have been published regarding the administration of enrofloxacin for the prevention of transportation-associated fever. The purpose of the study reported here was to evaluate effects of a single dose of enrofloxacin (5 mg/kg, IV) on body temperature and tracheobronchial neutrophil count in healthy Thoroughbreds premedicated with interferon-α and undergoing long-distance transportation.

Materials and Methods

Animals—Thirty-two healthy Thoroughbreds (16 males and 16 females; age, 2 years) were randomly selected for enrollment in the study. Horses were purchased for resale in the following year's trained-horse sales and had undergone race training for approximately 6 months at the Hidaka Training and Research Center of the Japan Racing Association in Hokkaido Prefecture. They were destined for the trained-horse sale held at the Japan Racing Association's Nakayama Racecourse in Chiba Prefecture. None of the horses had clinical signs of respiratory diseases such as pneumonia or pleuropneumonia. The horses' diet consisted of oats and sweet feed, which was the same as that fed during transport. All procedures were performed in accordance with the Japan Racing Association's institutional animal care and use committee rules.

Experimental design—Interferon-α[a] (0.5 U/kg, sublingually, q 24 h) was given to all horses in the study on 2 consecutive days before transportation and on the day of transportation.[11,12] Horses were randomly assigned to receive enrofloxacin[b] (5 mg/kg, IV, once; enrofloxacin group) or saline (0.9% NaCl) solution (50 mL, IV, once; control group) ≤ 1 hour before being transported in groups via commercial vans for a 26-hour period. Each group comprised 16 horses (8 males and 8 females). A physical examination and hematologic analysis were performed for all horses before and after transportation. Moreover, 12 horses (6/group [3 males and 3 females]) were randomly selected for evaluation of a TBA before and after transportation. After transportation, all healthy nonfebrile horses underwent race training for 7 days.

Clinical examination and hematologic analysis—Before, during, and after transportation, the horses were observed and palpated for evidence of any locomotion problems, signs referable to the digestive tract, or other signs potentially associated with administration of interferon-α, enrofloxacin, or saline solution. The heart and lungs of horses were auscultated during physical examinations before and after transportation. Horses were weighed with a calibrated scale, and the condition of their feces was assessed prior to and after transportation. Rectal temperature was measured with a mercury thermometer immediately before and after transportation. A temperature < 38.5°C was considered normal.

Blood samples (10 mL) were collected from a jugular vein into plain blood-collection tubes[c] and tubes containing EDTA.[d] Blood collected in the EDTA-containing tube was used to determine WBC count and hemoglobin concentration with an automatic hemacytometer.[e] It was also used to measure Hct with a capillary tube and to calculate the N:L after the preparation of smears with a fixation and stain kit.[f] Two hundred WBCs were counted by use of a light microscope at 100× magnification, and the N:L was calculated.

Serum was separated from the samples collected in the plain blood collection tubes by centrifugation (2,000 × *g* for 10 minutes at 25°C). The SAA concentration was measured via a latex agglomeration method[g] by use of equine standard serum samples (obtained from the one of the study horse samples) with SAA concentrations ranging from 0.0 to 400.0 μg/mL; these standards were produced according to methods described in previous reports.[9] All blood and serum analyses were performed in a blinded manner by one of the authors (SH).

Collection and analysis of TBAs—Tracheobronchial aspirates were obtained 3 days before transportation (before the administration of interferon-α)

and 1 hour after arrival. Collection of a TBA was performed without sedation, and restraint was provided by application of a nose twitch for a short time. The TBA was collected in a sterile manner with 30 mL of sterile saline solution via a videoendoscope[h] forceps channel.[17] Before each TBA collection, the outer surfaces and the forceps channel of the videoendoscope were cleaned with benzalkonium chloride solution, 50% isopropanol, and sterile water. White blood cells in the TBA were quantified manually. Slide preparations were made by cytocentrifugation[i] according to the manufacturer's instructions with 1 mL of sample in manufacturer-supplied centrifugation solution, and samples were treated with manufacturer-supplied cytologic fixative solution. The prepared slide was stained with a modified Wright-Giemsa stain,[f] and differential cell counting was performed. Two hundred WBCs were counted by use of a light microscope at 100× magnification, and the percentage of neutrophils was calculated.[17] All counts were performed in a blinded manner by one of the authors (SH).

Transportation—The transportation route was from the training and research facility in Hokkaido prefecture to the racecourse in Chiba prefecture. The duration was approximately 26 hours, and the distance was 1,210 km. A large ferry was used for part of the trip (from Hakodate Port in Hokkaido prefecture to Aomori Port in Aomori prefecture); duration of the ferry portion of the journey was 3 hours 30 minutes.

Ten commercial vans were used, each of which could be loaded with 6 horses and was designed exclusively for equine transportation. The horses were fed twice during transportation (at comfort stops 7 hours and 23 hours after the start of transportation); they were individually provided with assorted types of sweet feed and oats (1 kg/horse) at each feeding. Water was available at all times, but hay was not provided. Travel breaks (approx 15 to 30 minutes) were taken every 4 hours, and the insides of the trucks were ventilated by opening windows at every break.

Treatment of horses with transportation-associated fever—Horses with rectal temperatures ≥ 38.5°C after transportation were treated with antimicrobials if deemed necessary by attending clinicians (including the authors, who had abundant experience in horse transportation) after clinical evaluation. Clinicians were blinded to group assignment of the horses at the time of this evaluation. In accordance with the clinical signs, horses were administered a penicillin-streptomycin combination[j] approved for use in horses in Japan (penicillin [8,000 U/kg] and streptomycin [10 mg/kg], IM, q 24 h) or cephalothin sodium[k] (20 mg/kg, IV, q 6 h) as treatment for transportation-associated fever. The penicillin-streptomycin solution was administered to horses that had a rectal temperature between 38.5° and 39.0°C without apparent signs of systemic or respiratory disease, and cephalothin sodium was administered to horses that had a rectal temperature > 39.1°C and appeared lethargic or had apparent respiratory signs such as coughing. These antimicrobials were selected in accordance with the susceptibility of the bacteria isolated from the respiratory tract of horses with lower respiratory tract infections following transportation in earlier studies.[1,5]

Statistical analysis—Data are expressed as mean ± SD. For analysis of measurement data, comparisons were made between groups via a Mann-Whitney *U* test performed with commercially available statistical software.[l] Values of $P < 0.05$ were considered significant.

Results

No adverse effects of experimental treatment, including evidence of locomotion problems or clinical signs referable to the digestive system, were detected in horses following administration of interferon-α, enrofloxacin, or saline solution before, during, or after transportation. No significant differences were found between the enrofloxacin and control groups in the various measurement values before transportation (**Table 1**).

Table 1—Mean ± SD values for variables evaluated in 32 healthy Thoroughbreds that received enrofloxacin (5 mg/kg, IV, once; enrofloxacin group) or saline (0.9% NaCl) solution (50 mL, IV, once; control group) ≤ 1 hour before long-distance transportation (duration, approx 26 hours).

	Group			
	Enrofloxacin (n = 16)		Control (n = 16)	
Variable	Before transportation	After transportation	Before transportation	After transportation
Body weight (kg)	492.4 ± 27.2	477.3 ± 25.7	488.4 ± 24.9	470.9 ± 24.0
Rectal temperature (°C)	38.1 ± 0.3	38.0 ± 0.2[a]	37.9 ± 0.2	38.5 ± 0.4[b]
Peripheral blood				
WBC count (No./mm^3)	8,150 ± 965	8,225 ± 904[c]	8,294 ± 1,722	10,825 ± 2,256[d]
N:L	1.5 ± 0.5	1.6 ± 0.4[c]	1.5 ± 0.7	4.3 ± 2.8[d]
SAA concentration (μg/mL)	0.2 ± 0.2	0.6 ± 1.6[c]	0.1 ± 0.1	63.4 ± 82.6[d]
Hct (%)	39.5 ± 3.5	42.3 ± 2.8	39.5 ± 2.5	41.3 ± 3.7
Hemoglobin concentration (g/dL)	13.6 ± 1.2	14.6 ± 1.0	13.5 ± 0.9	14.1 ± 1.4
TBA				
WBC count (× 10^5/mL)	1.3 ± 0.5	1.3 ± 0.5[c]	1.3 ± 0.5	14.5 ± 11.1[d]
Neutrophils (%)	2.7 ± 1.2	2.8 ± 1.0[c]	2.5 ± 0.8	67.0 ± 47.3[d]

All horses were premedicated with interferon-α for 3 days (2 days before transportation and on the day of transportation).

[a,b]Within a row, values with different superscript letters are significantly ($P < 0.05$) different. [c,d]Within a row, values with different superscript letters are significantly ($P < 0.01$) different.

Mean rectal temperature after transportation was significantly ($P < 0.05$) lower in the enrofloxacin group (38.1 ± 0.3°C) than in the control group (38.5 ± 0.4°C; Table 1). Three horses in the enrofloxacin group had body temperatures ≥ 38.5°C, and 2 were administered penicillin-streptomycin solution, whereas 7 of 9 horses in the control group with body temperatures ≥ 38.5°C received additional antimicrobial treatment; 3 of these received cephalothin sodium, and 4 received penicillin-streptomycin solution. Although rectal temperature in horses that were administered penicillin-streptomycin solution were approximately normal (range, 38.0° to 38.4°C) after a single dose, the 3 horses in the control group that received cephalothin sodium required a mean of 4 ± 2 doses before this was achieved.

After transportation, the WBC count ($P < 0.01$), N:L ($P < 0.01$), and SAA concentration ($P < 0.01$) in peripheral blood samples and the WBC count ($P < 0.01$) and neutrophil percentage ($P < 0.01$) in TBAs were significantly lower in the enrofloxacin group than in the control group (Table 1). In contrast, Hct and hemoglobin concentration in blood samples and body weight after transportation did not differ significantly between the 2 groups.

Discussion

On the basis of information obtained in studies[18,19] in which equine cells were treated with high concentrations of enrofloxacin in vitro, administration of high doses of this drug to horses has the potential to disrupt proteoglycan synthesis in articular cartilage. However, there were no reports of adverse effects in studies[14,20–22] in which enrofloxacin was administered to horses at doses of 1.25 to 7.5 mg/kg. In the study reported here, enrofloxacin (5 mg/kg, IV, once) or saline solution (50 mL, IV, once) was administered to healthy Thoroughbreds that were pretreated for 3 days with interferon-α (0.5 U/kg, sublingually, q 24 h) as an immunologic stimulant. No adverse effects, including problems with locomotion or clinical signs referable to the digestive system, were associated with the treatments before, during, or after long-distance transportation (duration, approx 26 hours).

Rectal temperature after transportation, WBC count, and N:L in the peripheral blood, together with WBC count and percentage of neutrophils in TBAs, were significantly lower in the enrofloxacin group than in the control group. In the enrofloxacin group, 3 of 16 horses were febrile (rectal temperature, ≥ 38.5°C) after transportation, compared with 9 of 16 control horses. A penicillin-streptomycin combination is authorized for use in horses in Japan and is routinely administered to horses at the dose used in the present study. On the basis of clinicians' decisions to administer the penicillin-streptomycin combination or cephatholin sodium to febrile horses after transportation, the clinical signs were subjectively milder in horses of the enrofloxacin group.

In previous studies,[3,6] fever associated with transportation was primarily attributed to infection with *S equi* subsp *zooepidemicus*. Although assessment of microbial populations was not performed in the present study, the results described here, when taken together with those of the previous studies,[3,6] appear to support the hypothesis that administration of enrofloxacin immediately prior to long-distance transportation may reduce infection in the bronchoalveolar region by opportunistic bacteria such as *S equi* subsp *zooepidemicus* that are normally present in the tonsillar tissues and trachea.

There have been 2 studies[10,11] of the efficacy of interferon-α administration prior to long-distance transportation. In 1 study[11] of young horses, the incidence of transportation-associated fever was significantly reduced in horses that received this treatment for 3 days prior to transportation, compared with horses that received no treatment. In the other study,[10] although interferon-α administration did not significantly decrease the incidence of transportation-associated fever in racehorses, it significantly reduced inflammation and improved subjectively assessed clinical condition, compared with findings in control horses. In the present study, we administered interferon-α to all study horses at a dosage similar to those used in those earlier studies, but found significantly ($P < 0.05$) lower values for mean rectal temperature; WBC count, N:L, and SAA concentration in peripheral blood samples; and WBC count and neutrophil percentage in TBAs in the enrofloxacin group than in the control group. These findings clearly indicate that enrofloxacin administered to interferon-α–treated horses ≤ 1 hour before transportation is more effective for the prevention of transportation-associated fever than is interferon-α alone.

The use of enrofloxacin raises concerns regarding the emergence of antimicrobial-resistant bacteria, and it is important that antimicrobials such as enrofloxacin are not used inappropriately.[20,21] The guidelines for enrofloxacin use in the Japan Racing Association's medical office require that it is only administered as prophylaxis against transportation-associated fever when the duration of transportation is expected to be ≥ 20 hours and the horse has had clinical signs of transportation-associated fever before or is considered to be at risk for developing transportation-associated fever (eg, if the horse has undergone laryngoplasty or has a history of pneumonia). Also, antimicrobial susceptibility tests of bacteria in the TBAs and fecal samples after transportation will be performed regularly in the future to monitor for emergence of enrofloxacin-resistant bacteria.

a. Baytril 5% injection, Bayer Health Care Japan, Kita-ku, Osaka, Japan.
b. Bimuron, Biovet, Shibuya-ku, Tokyo, Japan.
c. VP-P100K, Terumo, Shibuya-ku, Tokyo, Japan.
d. VC-C50, Terumo, Shibuya-ku, Tokyo, Japan.
e. K-4500 automatic hemacytometer, Sysmex, Kobe-shi, Hyogo, Japan.
f. Diff-Quick 16920, Sysmex, Kobe-shi, Hyogo, Japan.
g. Latex agglomeration method LZ test, Eiken SAA, Eiken Chemical, Taito-ku, Tokyo, Japan.
h. VQ8303A videoendoscope, Olympus Corp, Shinjyuku-ku, Tokyo, Japan.
i. Shandon Cytospin 4 cytocentrifuge, Thermo Electron Corp, Marietta, Ohio.
j. Mycillin solution, Meiji Seika Ltd, Chuo-ku, Tokyo, Japan.
k. Coaxin, Chemix Co Ltd, Yokohama-shi, Japan.
l. StatView, version 5.0, SAS Institute Inc, Cary, NC.

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