Transferability of Cephalothin to the Alveolar Cavity in Thoroughbreds

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ABSTRACT. Five Thoroughbreds were classified into 4 groups according to the administration method used for saline solution (saline), ambroxol, and cephalothin sodium (cephalothin). In group A, cephalothin was injected intravenously after oral administration of ambroxol. In group B, cephalothin was injected intravenously after oral administration of saline. Groups C and D were used as control groups. The dose of cephalothin or ambroxol was clinically administrated. Venous blood and bronchoalveolar lavage fluid (BALF) were sampled from each group. In groups A and B, cephalothin concentrations in plasma reached their maximum level 5 min after cephalothin administration and then declined over time. In plasma obtained from groups A and B, there were no significant differences in pharmacokinetic parameters ($T_{1/2}$, Kel, Vd). By contrast, cephalothin concentrations in BALF reached their peak at 180 min after cephalothin administration in both groups A and B and maintained a relatively high level even after 300 min. These findings indicate that cephalothin requires a relatively long period of time to move from the blood stream to the alveolar cavity, but once transferred to the alveolar cavity, it is preserved for a long time. In groups A and B, cephalothin concentrations in BALF were approximately at the same level. However, in group A, total protein in BALF was lower at 60, 180, and 300 min than the other groups. Then, cephalothin concentration was adjusted to total protein in BALF. After adjustment to total protein in BALF, group A showed a concentration level of cephalothin approximately 1.5-fold higher than that of group B. This suggests that the transferability of cephalothin to the alveolar cavity improves as a result of the oral administration of ambroxol.—**KEY WORDS:** ambroxol, bronchoalveolar lavage, cephalothin, Thoroughbred.

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Since respiratory disorders, especially lung disease, affect the athletic capacity of racehorses to a substantial degree and can even end their racing life, the diagnosis and treatment of such disorders require extreme care. In horses, as in humans, bronchoalveolar lavage plays an important role in the diagnosis of lung diseases [3, 6, 11]. Such diseases, especially bacterial infections, require adequate chemical therapy. During treatment, it is essential to select an antibacterial agent that has high transferability to the inflammatory bronchoalveolar cavity, together with an effective antibacterial spectrum against etiologic agents. Cephalothin sodium, the first generation cephem group, (cephalothin: Coaxin^{TM}, Tobishi, Tokyo) is clinically applied as an agent equipped with a broad spectrum of gram positive bacterium to gram negative bacterium for administration to many animals [4, 10, 12]. Currently there is no report regarding the transferability of this agent to the bronchoalveolar cavity. On the other hand, ambroxol, a respiratory tract surfactant-expectorant, is known to increase antibiotic levels in rat lungs through a mechanism there is still unclear [13].

The purpose of this study is to examine the transferability to the alveolar cavity of cephalothin, an antibacterial agent with a broad antibacterial spectrum, and the effects of the

preadministration of ambroxol on the transferability of cephalothin to the bronchoalveolar cavity.

MATERIALS AND METHODS

Horses: In this study, 5 Thoroughbreds (3 males, 2 females: 4 years old; 438.0 ± 17.2 (SD) kg, range 410 to 460 kg) with no history or clinical signs of respiratory tract disease were used.

Drugs: we used cephalothin, ambroxol (Mucosolvan^{TM}, Teijin, Tokyo), and 0.9% physiological saline (saline: physiological saline, Otsuka Pharmaceutical, Tokyo) in the control and as a solvent of cephalothin and ambroxol. Cephalothin and ambroxol were dissolved in 50 ml and 20 ml of saline, respectively. We have administrated cephalothin or ambroxol in clinical doses.

Administration methods: The experiment was performed 4 times on each horse over an interval of more than 1 week. Horses were divided into 4 groups. In group A, 120 min after oral administration of 300 mg of ambroxol, 10 g of cephalothin was injected intravenously to the right jugular for 1 min. In Group B, 120 min after oral administration of 20 ml of saline, 10 g of cephalothin was injected intravenously to the right jugular for 1 min. In group C (control), 120 min after oral administration of 300 mg of ambroxol, 50 ml of saline was injected intravenously to the right jugular for 1 min. And in group D (control), 120 min after oral administration of 20 ml of saline, 50 ml of saline was injected intravenously to the right jugular for 1 min. Horses in group A were used to determine the effect of the ambroxol to the concentration of cephalothin. Horses in

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group B were used to determine the concentration of cephalothin. Horses in groups C and D served as a control group to determine the degree of the dilution rates for BAL.

Sampling methods: Blood samples were collected from the left jugular before oral administration of ambroxol or saline (-120 min), before injection of cephalothin or saline (0 min), and 5, 15, 30, 60, 120, 180, 240, and 300 min after injection of cephalothin or saline. Samples of bronchoalveolar lavage fluid (BALF) were collected before oral administration of ambroxol or saline (-120 min), after injection of cephalothin or saline (0 min), and 60, 180, and 300 min after injection of cephalothin or saline, by the bronchoalveolar lavage method previously described [6]. Specifically, the horse was sedated with medetomidine (7.5 µg/kg, IV) [5]. An equine bronchoscope (GIF TYPE XQ 20 long type™, Olympus, Tokyo) was then inserted through the nostril to the sampling site while the respiratory tract mucosa were anesthetized with 2% lidocaine. The site was lavaged 3 times with 50 ml of 37°C saline. A total of 6 lavage sites were established at the anterior (1 site) and middle portions (2 sites) of the lower lobes of the right and left lung. One site was selected randomly for each sampling, but the same site was not selected twice in a single experiment.

Measurement method for cephalothin: The concentration of cephalothin was measured by a high-performance liquid chromatography (HPLC) method partly modified from the method employed by Brisson and Fournillan [2]. A Waters Assoc. liquid chromatograph was used (Waters 625 LC System™, Waters, U.S.A.). A Model 486 ultraviolet detector, set at 270 nm, was also used. The samples (1 ml) or standard solutions (1 ml) were mixed with 0.5 ml of 0.4 M hydrochloric acid in a 10-ml centrifuge tube. The mixture was extracted with 7 ml of chloroform-1-pentanol (3:1 v/v), the extraction time being 10 min. After centrifuging at 1,000 g, 5 ml of the organic phase were transferred into another centrifuge tube and cephalothin was back-extracted (10 min) into 350 µl of 10 mM phosphate buffer (pH 7.0). After centrifugation at 1,000 g, part of the aqueous phase was injected into the chromatographic column (Puresil C18 column™, Waters, U.S.A.) that had been equilibrated with 0.01 M acetate buffer and methanol at a ratio of 85 to 15. The cephalothin concentration was then measured and recorded at its peak.

Pharmacokinetic parameters: The pharmacokinetics of cephalothin were observed in groups A and B. The serum concentration as a function of time followed a one-compartment open model, and the pharmacokinetic parameters were calculated from the following equation:

$$T_{1/2} = 0.693 / K_{el}$$

$$V_d = D / C \text{ (l/kg)}$$

K_{el} is the elimination rate constant, V_d is the volume of distribution, D is the dose, and C is the serum concentration of antibiotics.

Measurement method for total protein: Total protein in

BALF was measured in accordance with the Bradford method [1], using horse albumin as a standard.

Statistical analysis: The ANOVA-multiple method, with a significant difference of $p < 0.05$, was used for the analysis of each group. The result was shown with mean $\pm$ SD.

RESULTS

Cephalothin concentration in plasma: In both groups A and B, cephalothin concentration in plasma reached approximately 170 µg/ml plasma at 5 min after administration of cephalothin (Fig. 1). Cephalothin was not detected in groups C and D. No significant difference was observed between groups A and B.

Pharmacokinetic parameters: The pharmacokinetic parameters of cephalothin were studied in groups A and B. Table 1 shows the values for $T_{1/2}$, K_{el} , and V_d of cephalothin. In plasma obtained from groups A and B, there is no significant difference in pharmacokinetic parameters.

Cephalothin concentration in BALF: Cephalothin concentration in BALF was above 37 µg/ml BALF in groups A and B after 60, 180, and 300 min, and reached its maximum level after 180 min (Fig. 2). No significant difference was observed between groups A and B. Cephalothin was not detected in groups C and D.

Total protein concentration in BALF: Total protein concentration in BALF remained at approximately 30 µg/ml BALF. In group A, the minimum values were obtained at 60 and 300 min after cephalothin administration (14.4 ± 10.0 and 17.4 ± 8.6 µg/ml BALF, respectively). No significant difference was observed among the groups (Fig. 3).

Cephalothin concentration's adjustment according to the total protein concentration in BALF: After cephalothin concentration in BALF was adjusted to correspond to the

Fig. 1. Changes in cephalothin concentration in plasma: In group A (○) and B (●), cephalothin concentration in plasma reached approximately 170 µg/ml plasma at 5 min after administration of cephalothin. Data are expressed as mean $\pm$ SD.

Table 1. Pharmacokinetic parameters of intravenous cephalothin

	Horse No.	T _{1/2} (hr)	Kel	Vd (l/kg)
Group A	1	0.73	0.95	0.25
	2	0.79	0.88	0.25
	3	0.82	0.85	0.25
	4	0.80	0.87	0.25
	5	0.78	0.89	0.25
	mean SD	0.78 0.03	0.89 0.04	0.25 0.00
Group B	1	0.75	0.92	0.25
	2	0.78	0.89	0.25
	3	0.79	0.88	0.25
	4	0.76	0.91	0.25
	5	0.73	0.95	0.25
	mean SD	0.76 0.02	0.91 0.02	0.25 0.00

Fig. 2. Changes in cephalothin concentration in BALF: Cephalothin concentration in BALF was above 37 $\mu\text{g/ml}$ BALF in group A (○) and B (●) after 60, 180, and 300 min, reaching a maximum level after 180 min. Data are expressed as mean $\pm$ SD.

total protein levels in BALF, group B showed a value of approximately 2 $\mu\text{g}/\mu\text{g}$ protein, while group A demonstrated a level of approximately 3–4 $\mu\text{g}/\mu\text{g}$ protein, approximately 1.5 fold higher than that of group B (Fig. 4).

DISCUSSION

In selecting a drug for the treatment of respiratory disorders, especially bacterial infections, it is vital to use an antibiotic with an effective antibacterial spectrum as well as high transferability to the inflamed bronchoalveolar cavity. In human respiratory disorders, the transferability of antibiotics to the bronchoalveolar cavity has been assessed by measuring their concentrations in sputum, bronchial secretions, biopsy specimens of respiratory tract mucosal tissue, and excised pulmonary tissues [7–9]. Sputum, however, is accompanied by an intermixture of upper

Fig. 3. Changes in total protein in BALF: Total protein in BALF remained at about 30 $\mu\text{g/ml}$ BALF. In group A (○), the minimum value was reached 60 and 300 min after cephalothin administration. No significant difference was observed among the groups. Data are expressed as mean $\pm$ SD. ○: group B, ●: group C, ▲: group D.

Fig. 4. Changes of cephalothin concentration, adjusted to total protein concentration in BALF: When cephalothin concentration in BALF was adjusted to total protein concentration in BALF, group B (●) showed a value of approximately 2 $\mu\text{g}/\mu\text{g}$ protein, while group A (○) demonstrated a level of approximately 3–4 $\mu\text{g}/\mu\text{g}$ protein, approximately 1.5-fold higher than that of group B. Data are expressed as mean $\pm$ SD.

respiratory tract secretions and saliva. A high concentration of sputum is also prone to water reabsorption. Bronchial secretions, on the other hand, are intermixed with secretions from the upper-airway tract, lower-airway tract and alveoli, thus preventing correct evaluation of data from the bronchoalveolar cavity. For respiratory tract mucosal tissue biopsies, there were only a limited number of cases in which specimens could be obtained. These specimens were too small in size, and biopsy also suffers from fluctuations in concentration attributable to its weight. Furthermore, there were only a limited number of lung biopsy cases from which specimens could be obtained. BAL, by contrast, can recover BALF by selecting segmental bronchus under direct

broncoscopic observation. For these reasons, we examined the transferability of cephalothin to the bronchoalveolar cavity by using BALF.

Until now, publications and communications about minimum inhibitory concentration of cephalothin in horses have been nonexistent. Therefore, for lack of any recommended doses, we have administered cephalothin in clinical doses. Cephalothin concentration in plasma showed its peak value in both groups A and B at 5 min after cephalothin administration and then declined over time. By comparison, cephalothin concentration in BALF reached a peak value 180 min after cephalothin administration in both groups A and B, maintaining a relatively high level even after 300 min. These findings indicate that although the transfer of cephalothin from the blood stream to the alveolar cavity took a relatively long period of time, the high concentrations of cephalothin in the alveolar cavity were maintained even after cephalothin had nearly disappeared completely from the blood stream. These findings suggest that cephalothin is useful to the performance of antibiotics in the alveolar cavity, though the mechanisms of transferability still unclear.

Both groups A and B showed almost the same level of cephalothin concentration in BALF. Nevertheless, in groups B, C and D, no differences in total protein concentration in BALF were observed among the groups. In group A, however, total protein in BALF was lower at 60, 180 and 300 min than the other groups. These results suggest that groups A and B have different dilution rates for bronchoalveolar cavity elements. Next, cephalothin concentration was adjusted to protein concentration in BALF. After cephalothin concentration in BALF was adjusted to total protein in BALF, group A produced a concentration level of cephalothin approximately 1.5-fold higher than that of group B. This indicates that the oral administration of ambroxol enhanced the transferability of cephalothin to the alveolar cavity. It was not clear, however, how ambroxol affects transferability. Wiemeyer suggest that ambroxol enhances the permeability of the respiratory tract and the blood vessels [13], even though the details are currently unavailable. Further study will therefore be

required concerning this topic.

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