

## Concentration of cephalothin in body fluids and tissue samples of Thoroughbred horses

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*Cephalothin (CET) concentrations in body fluids (plasma, synovial fluid, pleural fluid, peritoneal fluid, and aqueous humor) and tissue samples (bone, lung, jejunum, hoof, and subcutaneous tissue) were investigated to consider the treatment of infectious diseases in horses. CET 22 mg/kg body weight was intravenously administered to 12 horses. Samples were collected from four different horses at 1, 3, and 5 hr after administration. The CET concentration in body fluids other than aqueous humor was maintained above the MIC<sub>90</sub> values of *Streptococcus zooepidemicus* and *Staphylococcus aureus* until 5 hr, but it was not maintained above that of *S. aureus* in bone. CET (22 mg/kg twice a day) is effective for septic arthritis, pleuritis, and peritonitis caused by gram-positive bacteria but ineffective for osteomyelitis.*

**Key words:** *body fluid, cephalosporins, pharmacokinetics, tissue*

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The first-generation cephalosporin cephalothin (CET) is effective against gram-positive bacteria and is used to treat equine infectious diseases [3, 5]. CET is metabolized to deacetylcephalothin, but deacetylcephalothin does not have a significant antibacterial effect compared with CET [9]. Plasma concentrations of CET have been reported in horses [9, 10, 16], but the penetration of CET into each part of the body has not been sufficiently evaluated. In this study, we investigated the concentrations of CET in body fluids and tissue samples to consider the treatment of infectious diseases at each site in horses.

For this study, 12 Thoroughbred horses (3–10 years old; 10 stallions and 2 mares) with body weights (bwts) of 446–532 kg were used. In all 12 horses, 22 mg/kg bwt of CET (Coaxin injection 1 g, Chemix Inc., Yokohama, Japan) was dissolved in 50 ml of sterile physiological saline and administered into the right jugular vein by bolus infusion. The dosage of 22 mg/kg was determined according to a

previous pharmacokinetic study [9]. Body fluids (plasma, synovial fluid of radiocarpal joint, pleural fluid, peritoneal fluid, and aqueous humor) and tissue samples (bone marrow of the proximal phalanx, lung, jejunum, subcutaneous tissue, and hoof, including the digital cushion, laminar layer, and sole dermis of the forelimb) were collected from four different horses at 1, 3, and 5 hr after administration. Plasma was collected immediately before euthanasia, and tissue samples and body fluids were collected immediately after euthanasia. The tissue samples were frozen immediately in liquid nitrogen and stored at –80°C until assay. Body fluids and plasma were immediately centrifuged at 1,500 g for 10 min, and the supernatants of body fluids and the separated plasma samples were stored at –80°C until analysis. From 2014 to 2018, these experiments were performed as the pathological anatomy of sarcoma or ataxia or as a secondary experiment with locomotive experiments including an experimental muscle injury model and a tendinitis model as primary experiments, which required euthanasia. All experiments were approved by the Animal Care and Use Committee of the Equine Research Institute of the Japan Racing Association (No. 17-9).

The quantitation of CET in plasma and body fluids was performed using a liquid chromatography system (Nexera X2, Shimadzu Corp., Kyoto, Japan) connected to a mass spectrometer (QTRAP 4500, SCIEX, Framingham, MA,

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USA), as previously described [9]. The tissue samples were ground into a frozen powder using a mixer mill frozen by liquid nitrogen (Retsch MM200, Verder Scientific GmbH & Co. KG, Haan, Germany). Next, 0.2 M phosphate buffer was added to 0.1 g of each tissue sample powder to create a 100 mg/ml solution. The solution was then sonicated using an ultrasonic bath (B 2200, Branson Ultrasonics, Brookfield, CT, USA) for 5 min and centrifuged at 12,000 rpm (4°C) for 5 min, and 500  $\mu$ l of the supernatant was mixed with 0.2 M phosphate buffer solution. The mixture was applied to a solid phase extraction cartridge (1 cc Oasis HLB, Waters Corp., Milford, CT, USA). The cartridge was washed with 1 ml of ammonium acetate (0.1 mM) and extracted with 1 ml of a mixture of 10 mM ammonium acetate and acetonitrile (7:3). Five microliters of eluate was injected into the same liquid chromatography-tandem mass spectrometry system as used for the quantitation of CET in plasma and body fluids. Quality control samples for calibration were prepared by adding standard CET (Toronto Research Chemicals, Toronto, Canada) to blank body fluids, plasma, and tissue samples that did not contain CET. The limits of quantification were 0.01  $\mu$ g/ml in plasma and body fluids and 0.01  $\mu$ g/g in tissues. The mean ( $\pm$  SD) extraction recoveries of CET were 83.5  $\pm$  2.9% for plasma, 76.2  $\pm$  9.2% for synovial fluid, 90.7  $\pm$  8.6% for pleural fluid, 87.9  $\pm$  3.0% for peritoneal fluid, 78.5  $\pm$  7.20% for aqueous humor, 98.5  $\pm$  6.2% for bone, 85.3  $\pm$  2.6% for lung, 83.9  $\pm$  2.6% for jejunum, 83.1  $\pm$  3.8% for subcutaneous tissue, 85.8  $\pm$  6.5% for digital cushion, 89.8  $\pm$  7.2% for lamina propria, and 95.0  $\pm$  11.5% for sole dermis (3 replicants). Statistical comparisons between body fluids and plasma concentrations were performed using the Mann-Whitney U test, with  $P < 0.05$  considered statistically significant.

The mean CET concentrations in the body fluids and plasma are shown in Fig. 1. The CET concentrations in the pleural and peritoneal fluids were significantly higher than that in the plasma at all time points and in the synovial fluid at 3 and 5 hr after administration. The CET concentration in the aqueous humor was significantly lower than that in the plasma at all time points. The mean CET concentrations in the tissue samples are shown in Fig. 2. The CET concentrations in the bone, hoof, and subcutaneous tissue samples were significantly higher than those in the lung and jejunum at 5 hr after administration.

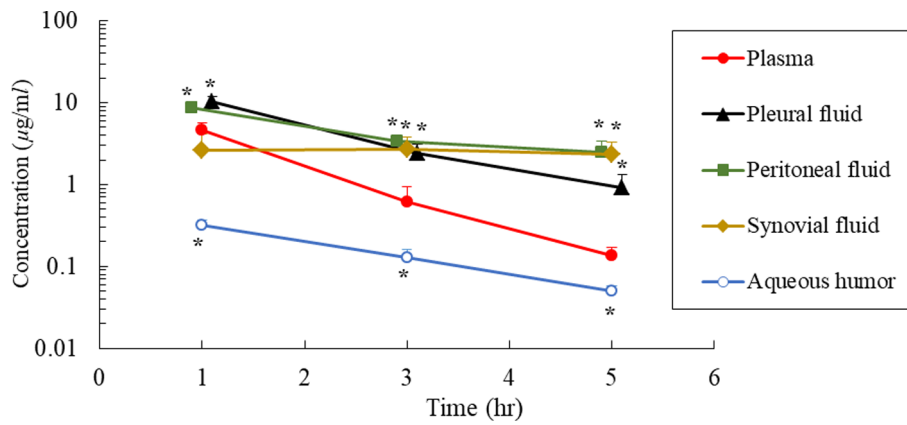
Since free drugs that do not bind to plasma proteins can penetrate the third space through vessels, a high free/total concentration ratio due to low protein binding in plasma is considered to lead to high penetration of body fluids [13]. Because a high free/total concentration ratio has been reported for CET (80.1–82.1%) in horses [9, 16], CET is considered to have good penetration into body fluids. Our study also indicated good penetration of CET into body

fluids.

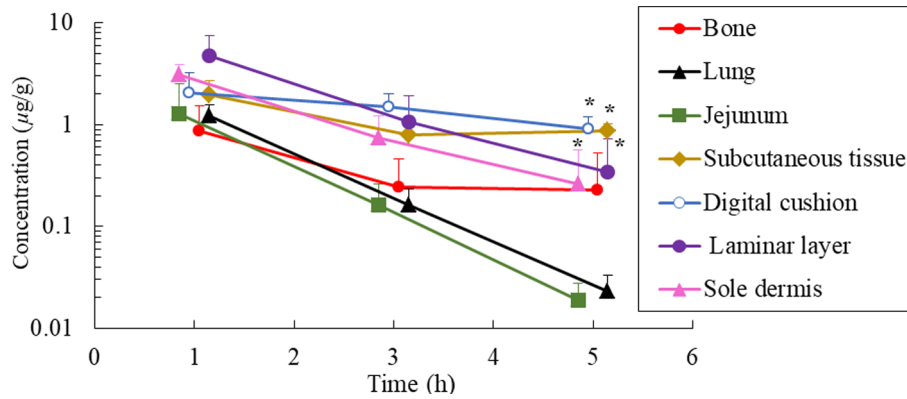
The antibacterial effect of the clinical dosage regimen was evaluated with a pharmacokinetic-pharmacodynamic (PK/PD) analysis [1]. Cephalosporins are time-dependent antimicrobials for which the appropriate PK/PD index is  $T_{>MIC}$  (the time during which the concentrations are above the minimum inhibitory concentration [MIC]), with a typical target value of 40% of the dosing interval [6]. This implies that if the concentration of a single dose exceeds the MIC for over 5 hr, it is considered to be effective when given twice a day. MICs of CET against bacteria isolated from horses have been reported previously, and the MIC<sub>90</sub> values against *Streptococcus zooepidemicus*, *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella* spp. were 0.12, 0.5, >4.0, and >4.0 mg/l, respectively [9]. When MICs and body fluid concentrations of CET were compared, body fluids other than aqueous humor maintained concentrations above the MIC<sub>90</sub> values of *S. zooepidemicus* and *S. aureus* until 5 hr after administration. This result indicates that administration of CET 22 mg/kg twice a day is expected to be effective against these infections in body fluids. *S. aureus* and *Streptococcus* species have been frequently isolated from equine limb infections [6], and *S. zooepidemicus* has been isolated from equine respiratory infections [19]. Our results indicate that CET (22 mg/kg twice a day) is effective for treating septic arthritis, pleuritis, and peritonitis caused by gram-positive bacteria. However, the CET concentrations of body fluids were not above the MIC<sub>90</sub> values of *E. coli* and *Klebsiella* spp. at all time points, suggesting that CET may not be effective for these infections in body fluids. Because it did not penetrate into the aqueous humor well due to the blood-aqueous barrier, CET was ineffective against anterior chamber infections.

In this study, the lung concentration of CET did not exceed the MIC<sub>90</sub> of *S. zooepidemicus*, which is considered a pathogenic bacterium for equine pneumonia [19]. However, since pathogens responsible for pneumonia are usually detected in epithelial lining fluid and bronchoalveolar lavage fluid, the CET concentrations of these fluids or the free plasma concentrations are more important targets for PK/PD analysis than the tissue concentration [12, 13]. Therefore, it may be inappropriate to consider the effect of CET on the lung tissue concentration. CET concentrations in bronchoalveolar lavage fluid of horses have been reported to be sufficient to control *S. zooepidemicus* [10].

The CET concentration was higher than the MIC<sub>90</sub> values of *S. zooepidemicus* and *S. aureus* until 5 hr in subcutaneous tissues and the digital cushion and until 3 hr in the lamina propria and sole dermis. Thus, CET administration twice or three times a day could control gram-positive bacteria that were frequently isolated from horses with cellulitis [6]. Administration of CET every 6–12 hr resulted in a high



**Fig. 1.** Mean (+ SD) CET concentrations in body fluids after administration of 22 mg/kg CET in Thoroughbred horses. Asterisks indicate statically significant differences ( $P<0.05$ ) between plasma concentrations and body fluid concentrations. CET, cephalothin



**Fig. 2.** Mean (+ SD) CET concentrations in tissue samples after administration of 22 mg/kg CET in Thoroughbred horse. Asterisks indicate statically significant differences ( $P<0.05$ ) compared with the lung and jejunum concentrations. CET, cephalothin

cure rate (94.7%) in 3,292 horses with limb infections [9]; our results for the CET concentrations in subcutaneous and hoof tissues are in line with the effectiveness of CET for treating limb infections.

Because of its unique structural properties, comparison of the bone concentration and MIC is considered important in osteomyelitis [12] and has been reported in humans [12, 17] and horses [4, 18]. *S. aureus* is the most common bacterium in osteomyelitis in humans [8] and animals [7]. The CET concentration in the bone marrow was lower than the MIC<sub>90</sub> of *S. aureus* at 3 and 5 hr after administration in this study, and our results indicate that osteomyelitis is difficult to control with systemic administration of CET. Local administration, such as regional limb perfusion and intraosseous administration, is recommended to reach the therapeutic concentration of other antimicrobials for osteomyelitis [14, 15].

The CET concentration in the jejunum was lower than that in foot tissues at 5 hr after administration in this study. There are no reports about the CET concentration in the jejunum and its effect on abdominal infectious diseases, but low concentrations may contribute to lesser effects on the intestinal microflora. Because antimicrobials cause disturbance in the microflora and increase the risk of diarrhea or enterocolitis in horses [2, 11], a low concentration in the jejunum is considered better for horses.

This study considered the treatment of infectious diseases at different sites in horses by evaluating CET concentrations in body fluids and tissue samples in adult Thoroughbred horses and the MIC<sub>90</sub> values of bacteria isolated from Thoroughbred racehorses with infectious diseases, including pneumonia and cellulitis, at two training facilities (Ritto and Miho training centers) in a previous report [9]. CET concentrations or MICs of bacteria may be different between this

report and reports for other populations, such as foals or horses in other facilities.

In this study, CET showed good penetration into body fluids, and a dose of 22 mg/kg twice a day was effective for septic arthritis, pleuritis, and peritonitis caused by gram-positive bacteria but ineffective for osteomyelitis due to an insufficient bone concentration.

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