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Intramuscular administration of sodium benzylpenicillin in horses as an alternative to procaine benzylpenicillin

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ABSTRACT

The aim was to supply information about the possibility of replacing the procaine salt with the sodium salt for benzylpenicillin IM treatment in horse in order to diminish the risk for procaine adverse effects. In a crossover study eight horses were given 15 mg/kg sodium benzylpenicillin (Na-pc) twice daily or procaine benzylpenicillin (control) once daily IM for four days. The half-life of Na-pc was 1.9 h, peak concentration was 14,600 ng/mL reached after about 23 min. Trough plasma concentration was 281 ng/mL and protein binding 62.8%. The *f*T > MIC for *Staphylococcus aureus* was 63% and 100% for *Streptococcus equi* subsp. *equi* and *Streptococcus zooepidemicus*, indicating an adequate antimicrobial therapy. However, Na-pc cannot be recommended from a welfare point of view since the horses showed more pain related behaviour and more pain and swelling compared to the control treatment.

1. Introduction

In Sweden benzylpenicillin is the most commonly used antibiotic in equine medicine. It is a safe drug but adverse effects sometimes occur. The adverse effects can be of different origin and different severity e.g. hypersensitivity or toxic reactions; mild, such as tremors, or nervousness, but can also be life threatening (Davis, 1987; Nielsen et al., 1988; Tjälve, 1991, 1997; Olsén et al., 2007).

In Sweden benzylpenicillin for use in animals is available both as sodium salt (Na-pc), in horses approved for IV injection, and as benzylpenicillin-procaine (pc-proc) for IM administration. Adverse effects after treatments of horses with pc-proc are commonly reported, and the procaine could be the cause of the majority of adverse reactions (Nielsen et al., 1988; Tjälve, 1997). In cattle Na-pc is approved both for intravenous (IV) and intramuscular (IM) administration and in swine only for IM administration. If Na-pc could be administered IM in horses the risk of procaine toxicity would be precluded. It would also enable the owners to continue the treatment IM after a veterinary visit. Another reason for considerations of a non-procaine based penicillin formulation is the occurrence of procaine positive test for performance horses.

Pc-proc is a depot preparation due to the low solubility of the procaine salt. The therapeutic benzylpenicillin-concentrations will be maintained for at least 24 h after IM administration (Stover et al., 1981; Sullins et al., 1984; Uboh et al., 2000) and the $t_{½}$ is 24.7 h (Uboh et al., 2000). After an IV. administration of Na-pc there is a rapid elimination of benzylpenicillin and the $t_{½}$ is about 50 min (Dürr, 1976; Love, et al., 1983).

Benzylpenicillin is a time-dependent antibiotic, implying the time the free serum concentration remains above the minimal inhibitory concentration (MIC) is most crucial for the antibacterial efficiency (Cars, 1997). Combining the pharmacokinetics (PK) with the pathogen susceptibility expressed by the MIC (used as a pharmacodynamic parameter, PD) is stated as efficacy indices or PK/PD-indices that are predictive for clinical cure. The PK/PD-index for benzylpenicillin correlating with efficacy is *f*T > MIC, i.e. the time the free concentration in plasma exceeds the MIC (Toutain et al., 2002). The breakpoint for clinical efficacy for benzylpenicillin is *f*T > MIC of 50–80% of the dosing interval (Toutain et al., 2002). *Streptococcus equi* subsp. *equi*, *Streptococcus zooepidemicus* and

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Staphylococcus aureus are all susceptible to penicillin and are common horse pathogens in Sweden, and hence were chosen as representative bacteria.

IM injections may lead to an inflammatory reaction with secondary swelling and muscle tenderness. Assessment of muscle tenderness can objectively be quantified using the pressure algometry technique and its registration of mechanical nociceptive threshold (MNT, measured in kPa) (Kosek et al., 1993; Chesterton et al., 2007; Chaves et al., 2010). Studies have shown that horses with a painful condition react earlier to the algometry provocation, thus showing a lower MNT than those without pain (Haussler and Erb, 2003, 2006; Varcoe-Cocks et al., 2006; Haussler et al., 2007).

Behaviour studies is a common way to assess pain in animals and are also previously used by our research team to measure effect of different drugs (Ingvast-Larsson et al., 2007, 2010, 2011).

The aim of this study was to investigate whether IM administration of Na-pc could be an alternative to pc-proc in horses. Various pharmacological characteristics were evaluated after repeated IM administration of Na-pc, such as pharmacokinetic parameters, the protein binding, and the effect of the drug measured as $fT > MIC$. To assess adverse effects such as inflammation, muscle tenderness and pain, the swelling and palpation pain was assessed with a clinical evaluation and registration of MNT at the injection site. Additionally, real time behavioural studies were performed. The horses' responses for both Na-pc and pc-proc (control) groups were evaluated.

2. Materials and methods

2.1. Horses and study design

Eight healthy standard bred trotters (seven mares and one gelding), 7–17 years old with a body weight of 471–581 kg were used. They were bedded on straw, and were fed hay and oats during the experiment. Water was available *ad libitum*. The study protocol was approved by the Animal Ethics Committee, Uppsala, Sweden (C8-88).

Each horse was given 15 mg/kg b.w. (approx. 25,000 IU/kg) Na-pc (Geepenil vet., Orion Pharma AB, Animal Health, Sweden, Na-pc 24 g, sodium citrate 1.1 g, injection concentration Na-pc 300 mg/mL, sodium citrate 14 mg/mL) every 12 h, seven times IM. Pc-proc, 21 mg/kg b.w. (approx. 21,000 IU/kg) (Penovet vet. Boehringer Ingelheim Vetmedica, Sweden, 1 mL contains 300 mg pc-proc and excipients: disodium phosphate dihydrate, povidone, lecithin, carmellose sodium, polysorbate 80, methylparahydroxybenzoate (E218), propyl (E216) and water for injection) every 24 h, was given IM four times. The study was in accordance with a 2-treatment, 2-period randomised cross over design. The wash out period was 19 weeks between the different drug treatment periods. Pc-proc was used as the control. The horses were IM injected in the left or right side of the neck every other administration, at 3 different sites.

During the study the horses were examined by clinical evaluation of the injection site, pressure algometry, behavioural studies, and blood samples were collected, all according to a specified schedule (described for each method).

2.2. Pharmacological assessments

2.2.1. Blood sampling

On the day before the start of drug administration, local anaesthetics lidocaine and prilocaine (Emla, ointment, lidocaine 25 mg/g, prilocaine 25 mg/g; AstraZeneca) were spread onto the skin covering one of the jugular veins after shaving and cleaning. About 60 min later, a catheter (14G, 13 cm Milacath, Mila International Inc., USA) was inserted into the vena jugularis. Blood samples (7 mL; sterile collected in heparinised test tubes and the catheter flushed with 0.9% NaCl after each sampling) were taken via this intravenous catheter at time 0 (pre-dose) and 0.25, 0.5, 1, 2, 3, 5, 8, 12, 24, 36, 48, 60, 72, 72.25, 72.5, 73, 74, 75, 77, 80, 84, 96, 108 and 120 h post administration. This implies that samples were taken immediately prior to the administrations at time-points 12, 24, 36, 48, 60 and 72 h (i.e. just before administration of a new dose) and also more frequently after the first (time 0) and the last (time 72) administration. All blood samples were collected into pre-chilled heparinised tubes, stored on ice water until centrifugation at 1500*g* for 10 min at 4 °C, and the plasma was separated and stored at −80 °C until analyses. Samples were collected from all horses at all time points, in order to treat every horse in a similar way, but only the blood samples from when the horses treated with Na-pc were analysed for benzylpenicillin concentrations.

2.2.2. Analysis of benzylpenicillin

Liquid chromatography tandem mass spectrometry (LC–MS/MS) was used for the determination of benzylpenicillin. 100 μL of internal standard solution (5000 ng benzylpenicillin-d7 free acid/mL in MilliQ-water: acetonitrile:methanol (2:1:1)) and 200 μL of MilliQ-water was added to 1.0 mL of plasma sample and mixed briefly. 3 mL of acetonitrile was then added, mixed and centrifuged for 10 min at 2000 g. The acetonitrile was evaporated from the supernatant under a stream of nitrogen at 40 °C, and then 100–200 μL was transferred to a micro-spin filter tube and centrifuged for 6 min at 11,000*g*. The sample (10 μL) was then injected into the LC–MS/MS system Finnigan TSQ Quantum Ultra (Thermo Electron Corporation). The ionisation mode used was negative electrospray. The injection was done through a guard column (C18, Size: 4 mm × 2.00 mm (length × inner diameter) and the separation was performed with an analytical column: Phenomenex Luna 5 μ C18 100R, 50 × 2.0 mm C18, Size: 4 mm × 2.00 mm (length × inner diameter). The chromatography was performed using a mobile phase consisting of a combination of 0.1% acetic acid with water and acetonitrile in a gradient program with a flow-rate of 200 μL/min. Data acquisition was with Selected Reaction Monitoring (SRM)/Multiple Reaction Monitoring (MRM): penicillin G: precursor m/z 333$[M-H]^-$ product m/z 192, penicillin G-d7: precursor m/z 340$[M-H]^-$ product m/z 199.

The quantification was carried out with calibration curves constructed by linear regression of the chromatographic peak area ratio (analyte/internal standard) as a function of analyte concentration. The calibration samples were prepared by spiking blank plasma with known amounts of analyte standard. For evaluation of the accuracy, the quantification precision as percentage relative standard deviation (RSD) and the linearity of the method quality control (QC) samples in three different concentrations were prepared by spiking blank plasma. The standards and the QC samples were treated in the same way as the plasma samples obtained from the horses given benzylpenicillin.

2.2.3. Pharmacokinetic (PK) analyses

Plasma concentrations of benzylpenicillin were plotted versus time for each horse, and the data were analysed using a commercially available software program (Win Nonlin 5.01, Pharsight Corporation). A non-compartmental model was applied for the analyses. The area under the plasma concentration time curve (AUC_{12h}) was calculated by the trapetzoidal rule from the time of dosing to 12 h post dosing after the first and last administration. The observed time (T_{max}) to maximal plasma concentration (C_{max}) and the C_{max} were read from the plotted concentration–time curve. Half-lives were determined from $t_{½}$ = ln2/terminal rate constant (λ). The trough plasma concentrations (C_{min}) were calculated from the plasma concentration values in blood samples taken immedi-