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Effect of repeated through-and-through joint lavage on serum amyloid A in synovial fluid from healthy horses

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ABSTRACT

The objective of this study was to evaluate the effect of through-and-through joint lavage on systemic and synovial serum amyloid A (SAA), total protein, nucleated cell count and percentage of neutrophils in the synovial fluid of six healthy horses. A prospective experimental study was performed where one healthy tarsocrural joint of each horse was randomly assigned to receive repeated through-and-through joint lavage at 0, 48 and 96 h. Synovial fluid and blood samples were collected at 0 (baseline), 24, 48, 72, 96 and 120 h. Systemic and synovial SAA, total protein, nucleated cell count and percentage of neutrophils were measured and compared to baseline. Concentrations of systemic and synovial SAA percentage of neutrophils were not increased from baseline in contrast to total protein and nucleated cell counts (except for nucleated cell count at 96 h). In conclusion, repeated through-and-through joint lavage did not affect synovial SAA concentrations in horses; however, synovial total protein and nucleated cell count values increased. Some of the total protein and nucleated cell count values observed in this study were within the range reported for septic arthritis 24 h after joint lavage. Hence, synovial SAA may be a valuable marker to evaluate the clinical progression of septic joints after through-and-through joint lavage. Clinical studies evaluating synovial fluid SAA concentrations while treating synovial sepsis with through-and-through joint lavage are warranted.

Introduction

Through-and-through joint lavage is a common procedure performed to treat infected joints in horses and is used as an alternative to arthroscopic lavage in the presence of increased anesthetic risk or economical constraints (Meijer et al., 2000; Milner et al., 2014). Through-and-through joint lavage may be repeated several times in the course of treatment as it is an easy procedure to perform and can be done in the standing patient (Meijer et al., 2000).

Practitioners usually rely on clinical evaluation (lameness, synovial effusion, heat, etc.) and sequential cytological examination of synovial fluid samples as a guideline for therapy adjustments and prognosis (Meijer et al., 2000; Walmsley et al., 2011). Reference values for the common synovial inflammatory markers in normal synovial fluid have been established as total protein of <25 g/L, nucleated cell count $<1 \times 10^9$ cells/L and neutrophils accounting for less than 10% of the total nucleated cells (Van Pelt, 1974). Sepsis has typically been considered with total protein >40 g/L, nucleated cell count $>30 \times 10^9$ cells/L and neutrophils >80% of the total nucleated cells (Van Pelt, 1974). However, other authors have suggested synovial sepsis with values of nucleated cell count $\geq 5 \times 10^9$ cells/L (Schneider et al., 1992; Milner et al., 2014). Unfortunately, horses with septic arthritis do not always show classical clinical signs of synovial infection (lameness, heat, synovial effusion, etc.) and the common synovial inflammatory markers can be altered in the absence of infection by procedures performed previously by the referring veterinarian (arthrocentesis, intra-articular administration of antimicrobials) or by procedures performed as part of the treatment (intra-articular administration of antimicrobials, arthroscopic lavage or through-and-through joint lavage), which complicate the interpretation of their clinical relevance (Bertone et al., 1986; Wilson et al., 1994; Klohnen et al., 1996; Dykgraaf et al., 2007; Sanchez Teran et al., 2012, 2014).

Serum amyloid A (SAA) in synovial fluid is a promising marker for septic arthritis as its concentrations are not affected by repeated arthrocenteses (Jacobsen et al., 2006b; Sanchez Teran et al., 2012), intra-articular administration of amikacin (Sanchez Teran et al., 2012) or arthroscopic lavage (Sanchez Teran et al., 2014) in healthy joints. SAA has been shown to increase from undetectable or very low concentrations in normal synovial fluid (<0.2–0.7 mg/L) (Jacobsen et al.,

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2006b; Sanchez Teran et al., 2012) to high synovial concentrations during septic arthritis (100–1500 mg/L) (Jacobsen et al., 2006b; Stack et al., 2014). The effects of repeated through-and-through joint lavage on SAA levels in equine joints are not known and these should be investigated before making recommendations regarding the clinical use of SAA in the assessment of septic synovitis in horses.

The objective of this study was to evaluate the effect of repeated through-and-through joint lavage on systemic and synovial fluid SAA concentrations, total protein, nucleated cell count and percentage of neutrophils in the synovial fluid from healthy equine tarsocrural joints. We hypothesized that systemic and synovial SAA would not change from baseline values after repeated through-and-through joint lavage while the other synovial parameters would increase significantly.

Materials and methods

Six healthy adult American Quarter horses (7 ± 3.5 years; 525 ± 64 kg) were used for this prospective experimental study. The University Animal Care and Use Committee approved all experimental procedures (Protocol number 20140010; issued on March 20, 2014). Horses were considered healthy and free of musculoskeletal pathology on the basis of results of a thorough physical examination, lameness examination, complete blood work (CBC and biochemistry profile including measurement of systemic blood SAA) and radiographic assessment of the tarsi (lateral, dorsopalmar, dorsolateral–palmaromedial oblique and dorsomedial–palmarolateral oblique views).

A tarsocrural joint in each horse was randomly assigned by a commercial statistics software program (Stata) to receive repeated through-and-through joint lavage at times 0, 48 and 96 h. Samples of synovial fluid were taken immediately before performing the joint lavage and at times 24, 72 and 120 h. Synovial fluid parameters measured at all time points included total protein, nucleated cell count, percentage of neutrophils and synovial SAA. A blood sample was collected in a serum tube by jugular venipuncture at all time points before arthrocentesis was performed. Serum and synovial fluid samples taken at 0 h were used as baseline values and served as controls for further statistical analysis. Physical and lameness examinations were performed every 24 h on all horses until the last sampling (120 h) and 7 days after the third lavage.

Horses were restrained in stocks and sedated with detomidine hydrochloride (0.02 mg/kg IV) and butorphanol tartrate at (0.03 mg/kg IV). The assigned tarsus was clipped and aseptically prepared. Prior to joint lavage, 3 mL of synovial fluid was collected from the dorsomedial pouch of the tarsocrural joint using a 22 G, 3.8 cm long needle (Moyer et al., 2007). The sample was then separated in two aliquots (1.5 mL each) and placed into two EDTA tubes.

Through-and-through joint lavage of the tarsocrural joint was performed after synovial collection using a 14 G, 3.8 cm long needle, inserted perpendicular to the skin into the dorsomedial pouch, medial to the saphenous vein and approximately 2.5 cm distal to the level of the palpable medial malleolus (Moyer et al., 2007). A second 14 G, 3.8 cm long needle was then inserted perpendicular to the skin into the dorsolateral pouch of the tarsocrural joint lateral to the extensor tendons just below the palpable lateral malleolus of the tibia. Sterile pump tubing was attached to the needle inserted in the dorsomedial joint pouch, and 3 L of Lactated Ringer's Solution (LRS) was infused using a peristaltic fluid pump (Spencer Varistaltic Dispenser and Pump, Manostat) at a flow setting of 150 mL/min. After lavage was performed, excess fluid was evacuated from the joint before retrieving the needles. Light bandages consisting of sterile non-adherent pad (Medline Industries Inc.), kling gauze (Covidien) and adhesive bandage (BSN Medical) were applied. The number of attempts to obtain a synovial fluid sample (needle replacements) was recorded, as well as any complication found while lavage was performed.

Sample analysis

A single clinical pathologist, who was blinded to times of collection and study protocol, examined one aliquot of the synovial fluid sample. Subjective assessment of color, clarity and presence or absence of solid material was performed. Samples were subjectively graded for blood contamination in a blinded fashion according to a scale from zero to four as follows: 0 = clear with no signs of blood contamination; 1 = slightly uniform hemorrhagic color; 2 = mildly hemorrhagic, mild uniform red color; 3 = moderately hemorrhagic, uniform red color but still translucent; and 4 = severely hemorrhagic, uniformly red and not translucent. Total protein was determined through refractometry (Reichert). A direct smear was made before the sample was treated with 0.1 mg of hyaluronidase (hyaluronidase from bovine testes; type VIII lyophilized powder 300–1000 U/mg; Sigma). Nucleated cell count was obtained using an automated hematology analyzer (Abbott Laboratories). A cytocentrifuge preparation (Shandon Southern Instruments) was prepared and stained using a Romanowsky stain (Fisher Scientific). Cytological examination was subjectively assessed for cellular preservation and presence of etiologic agents. A 100 cell differential count was performed on each sample using the cytocentrifuge preparation.

The second aliquot of synovial fluid and blood samples were centrifuged at 2100 *g* for 15 min (Beckman Coulter). Synovial fluid supernatant and serum were stored at −80 °C. When collection of all samples was finalized, synovial fluid and serum samples were thawed at room temperature. Ten microliters of hyaluronidase (Sigma; 500 U/mL) was added to 490 µL of synovial fluid to reduce its viscosity. Quantification of systemic and synovial SAA was performed on an automated chemistry analyzer (F. Hoffmann-La Roche) using a human SAA turbidometric immunoassay (Eiken Chemical). The assay has been previously validated for equine use reporting coefficients of variation at high, intermediate and low concentrations of SAA being 2.1, 1.6 and 24.4% respectively for intra-assay variation and 6.5, 4.6 and 33.2% respectively for inter-assay variation (Jacobsen et al., 2006a).

Statistical analysis

All data were analyzed with a commercial statistics software program (Stata 12IC, Stata). Descriptive statistics were used to summarize the distribution and central tendency of the independent and dependent variables. Many of the measured outcomes were not normally distributed and non-parametric statistics were used for evaluation. Quantile regression was used to compare the baseline median value of each of the outcomes at each sampling time point in separate models. The outcomes included total protein, nucleated cell count, percentage of neutrophils, systemic SAA and synovial SAA concentrations. The degree of blood contamination and needle replacements were individually tested as potential confounding variables for all outcomes in the quantile regression models. The overall significance of each confounding variable in the models was tested with an F-test. Significance level was set at $P < 0.05$.

Results

All horses remained healthy and did not show lameness throughout the study. Joint lavage was performed successfully in all horses with minor complications. These included movement of the horse during the procedure, which resulted in flexion of the limb and short interruption of the egress flow. The mean ± standard deviation (SD) time to infuse 3 L of LRS was 23 ± 3 min. Mild effusion of the treated tarsocrural joint was noticed after the first lavage in all horses and resolved within 7 days after performing the last joint lavage. The effusion was not associated with heat or pain on palpation.

When obtaining synovial fluid samples, replacement with a new 22 G needle occurred in 9/36 (25%) samples because of movement of the horse when sampling or insufficient synovial fluid quantity collected. When needle replacement was evaluated as a confounding variable, it was found to have no influence on the outcomes. Baseline samples were subjectively scored according to the amount of blood contamination as grade 4 in two samples, grade 2 in one sample, grade 1 in one sample and grade 0 in two samples. In the rest of the sampling times (not including baseline), blood contamination was subjectively graded 4 in seven samples, 3 in 11 samples, 2 in 11 samples, and 0 in one sample. Blood contamination score was found to be a confounding variable only to percentage of neutrophils ($r^2 = 0.44$, $P < 0.001$) and a higher blood contamination score was associated with a higher neutrophil count.

Total protein baseline values ranged from 10 to 25 g/L with a median of 12.5 g/L. Once repeated joint lavage was initiated, total protein values ranged from 15 to 60 g/L with a median of 26 g/L throughout the study. Concentrations of total protein peaked at 24 h after the first lavage and remained significantly increased compared to baseline at all time points (Table 1; Fig. 1a).

Nucleated cell counts followed a similar pattern to the one reported for total protein. Baseline values ranged from 0.1 to 0.5×10^9 cells/L with a median of 0.3×10^9 cells/L. During the rest of the study, nucleated cell count values ranged from 1.6 to 38.4×10^9 cells/L with a median of 4.7×10^9 cells/L. Values of nucleated cell count peaked at 24 h and were significantly higher than baseline values at all time points, except at 96 h ($P = 0.138$) (Table 1; Fig. 1b).

Baseline values for percentage of neutrophils ranged from 1 to 64% (median 23%). Throughout the study, values ranged from 21 to 78% with a median of 56%. There was no significant difference with baseline values ($P > 0.05$) when compared to the rest of the