Effects of Flunixin Meglumine, Metamizole and Phenylbutazone on
Equine Kidney Functions and Urinary Mucus and Immunoglobulin A (IgA) Secretions

Inaugural-Dissertation

to obtain the degree of a
Doctor medicinae veterinariae (Dr. med. vet.)
from the Faculty of Veterinary Medicine
Leipzig University

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Leipzig, 2019
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Day of defense: 26.03.2019
For my family and my friends
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<tr>
<td>BUN</td>
<td>Blood urea nitrogen</td>
</tr>
<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
</tr>
<tr>
<td>DIC</td>
<td>Disseminated intravascular coagulopathy</td>
</tr>
<tr>
<td>FE\textsubscript{Na}</td>
<td>Fractional excretion of sodium</td>
</tr>
<tr>
<td>FM</td>
<td>Flunixin meglumine</td>
</tr>
<tr>
<td>GGT</td>
<td>Gamma glutamyl transferase</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>GRF</td>
<td>Glomerular filtration rate</td>
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<tr>
<td>IL-1β</td>
<td>Interleukin-1β</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin-6</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
</tr>
<tr>
<td>MZ</td>
<td>Metamizole</td>
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<tr>
<td>NSAIDs</td>
<td>Nonsteroidal anti-inflammatory drugs</td>
</tr>
<tr>
<td>P</td>
<td>Phosphate</td>
</tr>
<tr>
<td>PGE</td>
<td>Prostaglandin E</td>
</tr>
<tr>
<td>PGI</td>
<td>Prostaglandin I</td>
</tr>
<tr>
<td>PHZ</td>
<td>Phenylbutazone</td>
</tr>
<tr>
<td>S-Cr</td>
<td>Serum creatinine</td>
</tr>
<tr>
<td>sIgA</td>
<td>Secretory immunoglobulin A</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor-α</td>
</tr>
<tr>
<td>TXA\textsubscript{2}</td>
<td>Thromboxane A\textsubscript{2}</td>
</tr>
<tr>
<td>U-Cr</td>
<td>Urinary creatinine</td>
</tr>
<tr>
<td>U-GGT</td>
<td>Urinary gamma glutamyl transferase</td>
</tr>
<tr>
<td>U-GGT:U-Cr</td>
<td>Urinary gamma glutamyl transferase: urinary creatinine ratio</td>
</tr>
<tr>
<td>UTIs</td>
<td>Urinary tract infections</td>
</tr>
<tr>
<td>UUN</td>
<td>Urinary urine nitrogen</td>
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1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most used drugs in equine medicine, mainly to manage inflammation, endotoxemia, pain and fever (COOK and BLIKSLAGER 2015). NSAIDs work by inhibiting cyclooxygenases (COX), particularly COX-1 and COX-2, which are responsible for synthesizing prostanoids, which they are important to prevent gastrointestinal (GI) tract ulcers, enhance GI tract mucosal repair, and control and maintain GI tract and renal blood flow to correct hypovolemia (TOMLINSON and BLIKSLAGER 2004; MCCONNICO et al. 2008; COOK and BLIKSLAGER 2015). By inhibiting COX enzymes, the NSAIDs can subsequently cause side effects such as GI and urinary tracts ulcerations, right dorsal colitis, diarrhea in foals and renal papillary necrosis (MACALLISTER et al. 1993; MCCONNICO et al. 2008; COOK and BLIKSLAGER 2015). Renal papillary necrosis can be caused by overdose in healthy horses or recommended dose of phenylbutazone (PHZ) in water deprived horses. The PHZ inhibits local synthesis of prostaglandin E$_2$ (PGE$_2$) and prostaglandin I$_2$ (PGI$_2$), which in well hydrated patients can compensate the reduced renal blood flow to renal medulla by inducing vasodilatation, however, in dehydrated patients, even with recommended dose of PHZ, can lead to necrosis of renal papillae (GUNSON and SOMA 1983; MACALLISTER et al. 1993). Side effects such as neutrophilia, progressive increase in some of the serum values such as blood urea nitrogen (BUN), creatinine (S-Cr) and phosphate (P) have been observed in horses treated with large doses of PHZ (MACKAY et al. 1983). Urinary gamma glutamyl transferase (U-GGT):urinary creatinine ratio (U-GGT:U-Cr) and fractional excretion of sodium (FE$_{Na}$) can be used to detect the effect of PHZ on the kidney function (El-ASHKER et al. 2012). The urinary tract is usually exposed to the pathogens, urinary mucus, which is produced by renal pelvis, and IgA play an important role in protecting the urinary tract. The mucus layer of mucous membranes is a sufficient defense mechanism to prevent adhesion of bacteria to the urinary epithelial cells and clear them from the system (HANSSON 2012). Previous studies have shown that urinary tract infections (UTIs) in horses are associated with any lesions which interfere with urine flow or being able to cause urinary mucosal damage (SAULEZ et al. 2005, SQUINAS and BRITTON 2013). Recent reports have indicated different information regarding the ability of phenylbutazone to affect the protective mechanisms of the urinary system, while one report has shown that long-term use of phenylbutazone can cause ulcerative cystitis in horses, presumably
Introduction

by reducing prostaglandins (ALEMAN et al. 2011), another study has reported that using phenylbutazone for 7 days has no effect on COX-1 and COX-2 expression in bladder mucosa (NIETO et al. 2012). In a study performed on children urine, it has been found that secretory immunoglobulin A (IgA) concentration increased in individuals with UTIs, and can be used as a marker to identify type of UTIs (DEO and VAIDYA 2004).

Renal failure in horses can occur as a result of decreased renal perfusion (prerenal failure), or cellular damage of the glomeruli or the tubules or tubular obstruction or tubulointerstitial inflammatory process (renal failure), or renal failure can be caused by interfering or obstruction of the urine outflow (HALBMAYR and SCHUSSE 1999; SCHULZE et al. 2004). Prerenal failure is the most common type of renal failure in horses, it is caused by decreased cardiac output or/and increased renal vascular resistance. Causes of decreased cardiac output in horses include diarrhea, endotoxemia, acute blood loss due trauma, septic shock, and prolonged exercise, which can cause decreased renal blood flow and glomerular filtration rate, and subsequent azotemia and electrolytes imbalances (WALDRIDGE 2010). NSAIDs can also cause prerenal azotemia in dehydrated horses, as they inhibit renal prostaglandins, which help to compensate decreased renal blood flow as they are vasodilatory mediators, therefore, using NSAIDs in dehydrated patients may exacerbate renal hypoperfusion and subsequent azotemia (MACALLISTER et al. 1993).

As NSAIDs are commonly used in equine practices, and little is known about their effects on the urinary defense mechanisms, accordingly, the objective of the present study was to investigate the influences of using flunixin meglumine, metamizole, and phenylbutazone on different equine urinary parameters, urinary mucus and immunoglobulin A (IgA) concentrations in horses.
2. Literature

2.1. Physiology of Prostanoids in Different Organs

Prostanoids are synthesized from an unsaturated fatty acid, arachidonic acid, by prostaglandin G/H synthase and cyclooxygenases (COX), which exist in two different isoforms, COX-1 and COX-2, and they are expressed by most body cells. The end products of the prostanoids synthesis are prostaglandin D$_2$ (PGD$_2$) (in the mast cells, brain and respiratory tract), prostaglandin E$_2$, (PGE$_2$) (in kidney, brain, platelets and vascular smooth muscle fibers), prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) (in uterus, vascular smooth muscle fibers, respiratory airway and eye), prostacyclin (PGI$_2$) (in the endothelia, kidney, platelets and brain) and thromboxane A$_2$ (TXA$_2$) in platelets, macrophages, vascular smooth muscle fibers and kidney. Production of prostanoids increased significantly during the inflammatory processes mediated mainly by COX-2, with COX-1 participating in early phase of acute inflammation, while COX-2 mediates its products several hours after the inflammation begins. PGE$_2$ and PGI$_2$ are the most formed pro-inflammatory prostanoids, both of them increase edema formation and leukocytes infiltration by increasing blood flow (MCADAM et al. 2000; SMYTH et al. 2009). Prostanoids can cause pain in two different ways, peripherally or centrally. Cells of damaged tissues and infiltrated immune cells release many mediators such as cytokines, chemokines, bradykinins, prostaglandins (e.g. PGE$_2$ and PGI$_2$) and others. PGE$_2$ and PGI$_2$ decrease the peripheral threshold of nociceptor sensory neurons to stimuli. Centrally, the expression of COX-1 and COX-2 in the spinal cord, in response to peripheral pain, leads to release of PGE$_2$, PGF$_{2\alpha}$ and PGI$_2$ which amplify the central sensitization to pain (CHEN et al. 2013).

2.1.1. Gastrointestinal System

The main prostanoids produced by mammalian gastric mucosa are PGE$_2$ and PGI$_2$, and to lesser extent PGF$_{2\alpha}$, PGD$_2$ and TXA$_2$. The prostaglandins protect gastric mucosa by stimulating the mucus and bicarbonate secretion in the stomach, improving the mucosal phospholipid layer and reducing epithelial cells permeability to luminal acid. Prostaglandins E and I are powerful vasodilator, which increase blood flow to the gastric mucosa. COX-1 is responsible for maintaining blood flow in healthy mucosa, while COX-2 mediates synthesis of prostaglandins in cases of mucosal injury. Prostaglandins inhibit releasing a couple of inflammatory mediators that contribute to the mucosal injury, such as histamine, tumor necrosis factor-$\alpha$, platelet activating
factor from mast cells, releasing of tumor necrosis factor-α and interleukin-1 from macrophages, releasing of leukotriene B₄ and interleukin-8 from neutrophils. All these mediators have been proofed to be suppressed by PGE₂. Gastric ulcer healing is derived primarily from COX-2. Ability of prostaglandins to heal gastric ulcers has been attributed to their abilities to inhibit gastric acid secretion, increase blood flow to the ulcer margin (where the epithelial cells regeneration takes place), stimulating mucus and bicarbonate secretion and stimulating angiogenesis through vascular endothelial growth factor (WALLACE 2008). Prostaglandins regulate intestinal smooth muscles contractility. PGE₂ causes intestinal longitudinal smooth muscles contraction and circular muscles relaxation, while PGF₂α causes longitudinal and circular smooth muscle contractions (HOOGMOED et al. 2000). Prostaglandins are also important for intestinal mucosal barrier repair after ischemic injury. The first response to intestinal mucosal injury is contraction of the intestinal villi, which takes place in two phases, the first phase is mediated by enteric nervous system, the second phase is mediated by prostaglandins. The second response is epithelial restitution, which is not depended on the prostaglandins. However, the prostaglandins stimulate recruitment of the tight junctions to close the apical paracellular space of the epithelial cells in the third process of the repairing the intestinal mucosal damage (MARSHALL and BLIKSLAGER 2011).

2.1.2. Musculoskeletal System
Prostaglandins, PGE₂ in particular, stimulate osteoprogenitor proliferation and differentiation. Administration of prostaglandins in vivo increases periosteal and endosteal responses which increase bone mass (JEE and MA 1997; GENETOS et al. 2011). Prostaglandins inhibition delays bone fracture healing and can lead to non-unions (GIANNOUDIS et al. 2000; SIMON et al. 2002; BURD et al. 2003; GERSTENFELD and EINHORN 2004). Joint inflammation, injury, cells damage, vascular distention, exercise or stress cause prostaglandins release. Equine chondrocytes produce PGE₂ in vitro when they are subjected to adverse conditions. PGE₂ production in the joint is very rapid, with peaking 2-9 hours after onset (VAN DEN BOOM et al. 2005). It has been suggested that PGE₂ acts as a mediator of the inflammatory process and hyperalgesia, through vasodilatory effect, increasing vascular permeability, and sensitization of joint nociceptors in horses (PALMER and BERTONE 1994). PGE seems to be associated with increased metabolic activity in the cartilage and bone resorption associated with osteomyelitis, whereas PGF is chondro-protective (KALLINGS 1993). Proteoglycans are the main component
of the cartilage matrix. During osteoarthritis, the proteoglycans undergo degradation accompany by inability of chondrocytes to synthesize new matrix. In a study to assess the effect of oral administration of phenylbutazone (PHZ) for 14 days on synthesis of proteoglycans and chondrocytes inhibition by interleukin-1β (IL-1β) in articular cartilage explants showed significant decrease in proteoglycan synthesis in articular cartilage culture explants from healthy individuals to the same degree of that induced by in vitro IL-1β exposure (BELUCHE et al. 2001). In another experiment, using PHZ (4.4 mg/kg, PO, q12h, for 30 days) significantly decreased mineral apposition rate in the tibia and seemed to decrease the healing rate of the unicortical bone defects in horses (ROHDE et al. 2000).

2.1.3. Kidneys
Both COX-1 and COX-2 are expressed in the kidney, with COX-1 is expressed mainly by epithelia of the glomeruli, arteriolar endothelia, cortical and medullary collecting ducts and mesangial cells, while COX-2 is expressed in the cortical ascending limb cells, macula densa and medullary interstitial cells. PGE₂, PGI₂, PGF₂α and TXA₂ are the most abundant prostanoids in the kidney. Under normal conditions, prostanoids have little effects on renal blood flow and glomerular filtration rate (GRF). However, under some conditions which compromise arterial blood volume, such as dehydration, congestive heart failure, cirrhosis with ascites, and nephrotic syndrome, normal kidneys functions depend on the effect of the prostanoids to restore normal renal blood flow. In the cases of hypovolemia, the body produces catecholamines, angiotensin, and vasopressin which act as vasoconstrictors. The kidneys depend on the effects of prostanoids which act as vasodilators and maintain normal renal blood flow. Renal PGE₂ maintains GFR by vasodilating the afferent arteriole. It has been suggested that COX-2 derived prostanoids play important role in renal sodium excretion and maintaining blood pressure, therefore, using COX-2 selective inhibitors results in sodium retention and edema formation. Also, COX-2 inhibitors decrease renal medullary blood flow, which causes sodium retention and subsequent hypertension (HAO and BREYER 2008; SMYTH et al. 2009).

2.2 Effects of Nonsteroidal Anti-inflammatory Drugs (NSAIDs)
Nonsteroidal anti-inflammatory drugs (NSAIDs) have been used in human and veterinary medicines for their analgesic, anti-inflammatory and antiendotoxic properties. Prostanoids decrease pain threshold for neural conduction of the pain pathway, therefore increase the
sensation of pain. PGE$_2$ sensitizes nociceptors at the end of sensory neurons. COX inhibitors decrease pain through reducing prostanoids production in the spinal cord and subsequent decrease central nervous system sensitization. The emergence of COX-2 inhibitors helps to provide the analgesic effects and avoid inhibiting COX-1, however, because the overlap between COX-1 and COX-2 functions, questions about the efficacy of COX-2 inhibitors have been raised. One study showed that using meloxicam (COX-2 inhibitor) at dose of 0.6 mg/kg, iv, q12h, did not inhibit the intestinal mucosal repair, however, provided almost the same level of analgesia as flunixin meglumine (FM) (1.1 mg/kg, IV, q12h) when the experimental horses were judged by clinician and combined blinded pain score, however, provided significantly less analgesia when judged by blinded observer. In another study using etodolac (COX-2 inhibitor) (23 mg/kg, iv, q12h) inhibited the small intestinal mucosal repair, suggesting that no advantage of using etodolac over FM (1.1mg/kg, IV, q12h) in horses. In experimental study, a highly selective COX-2 inhibitor firocoxib (0.09 mg/kg, IV, q24h) and FM were used in horses with small intestinal strangulating obstruction, the study concluded that firocoxib provided the same level of analgesia as FM, and also the FM caused significant increase in intestinal permeability to lipopolysaccharide (LPS) (TOMLINSON et al. 2004; COOK et al. 2009; NAYLOR et al. 2014; COOK and BLIKSLAGER 2015). FM is the most common used NSAID to reduce signs of endotoxemia in horses. In early study, pretreatment with FM (1 mg/kg) reduced the clinical signs of induced endotoxemia compared with PHZ (2 mg/kg). Several other studies have explained the effects of FM on cardiovascular responses to endotoxemia. Endotoxin increases the prostanoids TXA$_2$, 6-keto PGF$_{1\alpha}$ and PGI$_2$. Pretreatment with FM has been found to prevent increase in these prostanoids by endotoxin. Although the role of FM in reducing the effects of inflammatory response of the endotoxemia by inhibiting the prostanoids, it has no effect to other inflammatory mediators such as tumor necrosis factor-$\alpha$ (TNF-$\alpha$) and interleukin-6 (IL-6) (BOTTOM et al. 1981; OLSON et al. 1985; MOORE et al. 1986; JACKMAN et al. 1994; BASKETT et al. 1997; COOK and BLIKSLAGER 2015).

2.3. Side Effects of Nonsteroidal Anti-inflammatory Drugs (NSAIDs)

Gastrointestinal ulceration is the most common side effect of using NSAID. It has been found that COX-1 was expressed in horses with normal nonglandular portion of the stomach and reduced in horses with ulcerated lesions in the gastric nonglandular portion, where COX-2 was only expressed in the ulcerated stomach, this study indicated that NSAIDs should not use in
horses with gastric ulcers. Using NSAIDs in patients with intestinal mucosal injury may delay their repair, as prostaglandins play an important role in intestinal injury repair. It has been found in a study where ischemia was in horses’ jejunum that FM delayed intestinal mucosal repair, but did not increase absorption of the LPS. However, using selective COX-2 inhibitors, such as firocoxib, meloxicam, did not delay the repair of the small intestinal ischemia. Other studies, on the effect of FM on recovery of the ischemia of colonic mucosa in horses, have found that the FM did not delay its repair (TOMLINSON and BLIKSLAGER 2004; LITTLE et al. 2007; COOK et al. 2009; MATYJASZEK, et al. 2009; RODRIGUES et al. 2010; MORTON et al. 2011; COOK and BLIKSLAGER 2015). The most NSAIDs associated with right dorsal colitis is PHZ, symptoms may include colic, diarrhea, and protein losing enteropathy. Hypoalbuminemia is one of the earliest symptoms, it can appear as early as 3 days after starting using PHZ. Transabdominal ultrasound can show increased mural thickness of the right dorsal colon. Neutropenia, changes in right dorsal colon arterial blood flow and changes in volatile fatty acids production have also been noticed (KARCHER et al. 1990; COHEN et al. 1995; JONES et al. 2003; MCCONNICO et al. 2008).

TXA₂ and prostacyclin are contributing to hemostasis in two opposite mechanisms. TXA₂ is synthesized by platelets, mediated only by COX-1. TXA₂ causes platelets aggregation and vasoconstriction. Prostacyclin is synthesized by endothelial cells, mediated by COX-2 and causes vasodilation and prevents platelets aggregation (PATRIGNANI et al. 1999; FOSSLIEN 2005; ROUBILLE et al. 2013). Several of studies in human medicine revealed the side effects of COX-2 selective NSAIDs, such as firocoxib, on the cardiovascular system, mainly because COX-2 inhibition shifts TXA₂-prostacyclin balance to the direction of TXA₂, which results in platelets aggregation and vasoconstriction. There are no documented cardiovascular side effects for PHZ and FM in horses. However, disseminated intravascular coagulopathy (DIC) is well documented in horses. DIC can be caused by different kinds of local or systemic inflamations, such as parasitic migration, colitis, small intestinal strangulation, and pleuropneumonia. Severe gastrointestinal disorders in horses usually results in fibrin deposits in different organs consistent with microthrombosis, multiorgan failure and DIC. Thromboembolism can occur in horses as jugular vein thrombosis, pulmonary arteries thrombosis, mesenteric veins thrombosis, or hepatic vein thrombosis. Aspirin, a COX-1 inhibitor, has been used for thromboprophylaxis in horses. Aspirin at 5 mg/kg, PO, q24h dose has significantly decreased serum thromboxane B₂ (TXB₂) in horses (COTOVIO et al. 2007; BRAINARD et al. 2011).
Foals are predisposed to NSAIDs’ side effects, probably because of different pharmacokinetics of NSAIDs compared to adults. Using PHZ at 2.2 mg/kg dose in foals has been found to have a longer half-life and a lower total clearance when compared to adults (WILCKE et al. 1993). In another study assessing the route of administration of FM in foals, using 1.1 mg/kg, po dose for 30 days caused oral ulceration, while those received the same dose intramuscular (im) did not show oral ulceration, however, both routes of administration caused gastric glandular ulcers (TRAUB-DARGATZ et al. 1988). In another experiment, different doses of FM (0.55 – 6.6 mg/kg for 5 days) were used in neonatal foals, diarrhea was the most common side effect, which was not dose-dependent, hypoproteinemia associated with gastrointestinal ulceration was also noticed, most of these ulcers were in the gastric glandular region (CARRICK et al. 1989). Study on oral meloxicam, using the same dose as adult horses (0.6 mg/kg, q12h), in foals has provided the same therapeutic levels with no side effects noted even at higher doses (1.8 mg/kg, q12h, for 7 days) (RAIDAL et al. 2013). Blood analysis, urinalysis, and gastroscopy did not show side effects in foals of 36 hours of age, when treated with firocoxib at dose 0.1 mg/kg, q24h, PO for 9 days (HOVANESSIAN et al. 2014).

Inhibition of renal PGI2 and PGE2 by using of NSAIDs in hydrated horses has little effects on renal perfusion. However, in dehydrated patients, inhibition of PGI2 and PGE2 result in vasoconstriction of the afferent arterioles, redirecting the blood flow to the renal cortex, and reduced or loss of renal medullary perfusion, which results in a characteristic lesion of renal papillary necrosis (READ 1983; MACALLISTER et al. 1993; WHELTON 1999; KIM 2008). PGE2 decreases water and sodium reabsorption at the thick ascending loop of Henle (EPSTEIN 2002), therefore, inhibition of PGE2 by NSAIDs would result on sodium retention. The effect of PHZ on the effect of furosemide on urinary electrolytes secretion has been assessed. PHZ significantly decreased urinary sodium and chloride excretion by 40% and 32% respectively when compared to patients treated with furosemide alone (DYKE et al. 1999). These findings should be taken into consideration when treating critically ill patients with both NSAIDs and sodium rich intravenous fluids for long time, as this combination might result in sodium retention and edema formation (COOK and BLIKSLAGER 2015). In human medicine, it has been found that using COX-2 selective NSAIDs is safer on the kidneys than nonselective NSAIDs. Long term using of celecoxib (a COX-2 selective NSAIDs) for patients with osteoarthritis or rheumatoid arthritis had significantly less renal toxicity than ibuprofen and diclofenac (nonselective NSAIDs) (SILVERSTEIN et al. 2000). No such study has been conducted to
evaluate the effect of COX-2 selective NSAIDs on the kidney in hypovolemic horses, therefore, to avoid nephrotoxicity, it is advisable to perform a complete physical examination to evaluate vascular volume through pulse strength, jugular fill, and volume and frequency of urination, then correct the intravascular volume prior to NSAIDs administration (COOK and BLIKSLAGER 2015).

2.4. Urinary Tract Infections (UTIs) and Defense Mechanisms

Urinary tract infections (UTIs) are caused by microbial invasion and colonization of the kidney, ureter, urinary bladder, and proximal urethra. UTIs can be divided into upper UTI, which involves the kidneys and ureters, and lower UTI, involving the urinary bladder and urethra. The incidence of UTIs is low in horses, particularly upper UTIs. Lower UTIs are the most common type of UTIs seen in horses, and usually caused by disrupted urine flow, mostly urinary stone or partial obstruction of the urinary tract. Escherichia coli, Proteus spp., Klebsiella spp., Enterobacter spp., Pseudomonas aeruginosa, Streptococcus equi subsp. zooepidemicus, and Streptococcus equisimilis are the most common bacteria involved in UTIs, and to a lesser extent Staphylococcus and Corynebacterium spp. Enterococcus spp. has been isolated from horses experiencing urine flow abnormalities or horses were urinary catheterized. It is common to isolate more than one bacterial species. Because of their shorter urethra and potential fecal contamination from poor perineal conformation, female horses are more prone to develop UTIs than male horses. Other UTIs’ risk factors include bladder paralysis, urolithiasis, urethral trauma. Cystitis may develop as a result of urolithiasis, bladder paralysis, or neoplasia. Urinary bladder paralysis can be caused by trauma or some neurologic diseases such as neuritis of the Cauda equine, equine protozoal myeloencephalitis or equine herpesvirus-1 (ZIMMEL 2014). One of the body’s defense mechanisms to protect itself against microbes and antigens is secretory immunoglobulin A (sIgA). sIgA is secreted in different body fluids, such as saliva, milk, gastrointestinal and respiratory secretions. Immunoglobulin A (IgA) is synthesized by plasma cells in the lamina propria of the mucous membranes or connective tissues of secretory glands. Some of these sIgA diffuse through the basement membrane into the epithelial cells, then transcytosed to the apical surface of the epithelial cells, then secreted as sIgA. sIgA in saliva is used as a stress marker and effects of stress on the immune system (TSUJITA and MORIMOTO 1999; CORTHÉSY 2013). It has been found that bronchial epithelial abnormalities lead to localized sIgA deficiency in chronic obstructive pulmonary disease (COPD) airways, which may
cause persistent airways inflammation (POLOSKUHIN et al. 2011). Intestines of human neonates are sIgA deficient and need around 30 days postpartum to develop the ability to produce effective levels of sIgA. The activation of intestinal plasma cells depends on intestinal colonization by \textit{Bifidobacteria} and \textit{Lactobacilli}. During this period, neonates depend on their mothers’ breast milk sIgA, which protects them against the surrounding environmental antigens such as toxins, microorganism, and dietary antigens (HE et al. 2007; WALKER 2010). Measuring sIgA in children’s urine can also be used to detect UTI, type of infection, and degree of the host’s response to the infection (DEO and VAIDYA 2004).

Equine urinary mucus is produced in renal pelvis and proximal ureter that helps to lubricate the lower urinary tract to minimize adherence of calcium carbonate crystal to the epithelium lining the ureters, bladder, and urethra which decreases the chances of urolithiasis and UTIs (WALDRIDGE 2010). Mucus is very important line of defense, as it removes and clears the potential microorganisms and antigens from the mucous membranes. In respiratory system, mucus-secreting ciliated cells produce the mucus and chloride pumps osmotically help the mucus hydration, then the cellular cilia move the mucus and the trapped antigens from the bronchi and trachea to the pharynx to be swallowed, the same mechanism takes place in the nose and paranasal sinuses, where the mucus being carried to the oropharynx to be swallowed (COHEN 2006; HARKEMA et al. 2006). Uterine epithelia provide a non-toxic mechanism to remove potential antigens and facilitating a suitable environment for early equine embryo by secreting sIgA to attach to the pathogens and trapped them in the uterine mucus, then cleared mechanically (CAUSEY 2007). In the stomach, the mucus’s main function is to protect the gastric mucosa acid and pepsin (ALLEN et al. 1986; KÖLLER et al. 2010), but also it prevents bacteria from adherence and potential translocation through the epithelial cells (KATAYAMA et al. 1997).
3. Results

3.1 Publication

Mohammed Adam, Gábor Köller, Corinna Arnold, Gerald F. Schusser

Effects of using Flunixin Meglumine, Metamizole, and Phenylbutazone on equine kidney functions, urinary mucus, and secretory Immunoglobulin A (IgA) concentrations

Pferdeheilkunde 2017;33:263-270.

Contribution:

The research project was planned by Mohammed Adam and Prof. Dr. Gerald Fritz Schusser.

The collection of study’s samples took place at the Department of Large Animal Medicine by veterinarians (Mohammed Adam, Dr. Corinna Arnold, and Prof. Dr. Gerald Fritz Schusser).

The collection and preparation of the patients’ data for the present study including classification and selection of the horses included in the study was carried out by Mohammed Adam. The collected data were statistically evaluated by Mohammed Adam and the results were processed as tables. The manuscript was written by Mohammed Adam, Prof. Dr. Gerald Fritz Schusser and Dr. Gábor Köller assisted as supervisors. Mohammed Adam contributed to the 95% of the publication.
Effects of using Flunixin Meglumine, Metamizole, and Phenylbutazone on equine kidney functions, urinary mucus, and secretory Immunoglobulin A (IgA) concentrations

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Summary: Nonsteroidal anti-inflammatory drugs (NSAIDs) are routinely used in equine practices to manage inflammation, endotoxemia, pain or fever in horses. This study was carried out to investigate the effects of the most commonly used nonsteroidal anti-inflammatory drugs on renal parameters, mucous and Immunoglobulin A (IgA) concentrations in horses. Thirty healthy horses (control group) at 20 horses with indications of using either flunixin meglumine (FM), metamizole or phenylbutazone (PHZ) have been used and assigned to group 1, 2 or 3, respectively. Serum creatinine, blood urea nitrogen and electrolytes, in addition to urinary creatinine (U-Cr), urine urea nitrogen and urinary electrolytes, were measured using an automatic analyser. Fractional excretion (Fe) of sodium, chloride, potassium, calcium, magnesium and inorganic phosphate, in addition to urinary protein (U-Pro) and urinary gamma glutamyl transferase (U-GGT) were calculated. Urinary mucus and IgA concentrations and their ratios to the urinary creatinine were measured. The FE was significantly higher in group 3 (P < 0.001) than the control group. The U-GGT/U-Cr ratio was also significantly increased in group 3 (P < 0.01) compared with the control group. The U-Pro/U-Cr ratio was significantly higher in groups 1 and 2 (P < 0.007 and P < 0.001, respectively) than in the control group. Phenylbutazone (PHZ) had a significantly increased mucus-U-Cr ratio (P < 0.005). Significant increases were observed regarding the IgA-U-Cr ratio in groups 1 (P < 0.007) and 2 (P < 0.014). In conclusion, long-term use of PHZ has an influence on the renal ascending limb of the loop of Henle, and all these drugs could have effects on the proximal tubules. Phenylbutazone causes an increase in urinary mucus secretion, probably as a protective mechanism against the necrotic effect of PHZ. Parameters such as U-Pro-U-Cr and U-GGT-U-Cr ratios and FE-IgA are helpful in detecting these renal abnormalities.

Keywords: flunixin meglumine, metamizole, phenylbutazone, kidney function, urinary mucus, immunoglobulin A (IgA), horse, equine

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are routinely used in equine practices to manage inflammation, endotoxemia, pain or fever in horses (Cook and Blikslager 2015). However, NSAIDs have been reported to have many side effects in horses (McAllister et al. 1993), human beings (Dahl et al. 2003), dogs (Kukuk et al. 2012) and cats (Lascar et al. 2007). In horses, the gastrointestinal (GI) tract and kidneys are considered the organs most affected by the side effects of NSAIDs. The mechanism of action of NSAIDs involves the inhibition of cyclooxygenases (COX), mainly COX-1 and COX-2. These enzymes are responsible for synthesizing prostanoids, which are important to prevent GI tract ulcers, enhance GI tract mucosal repair, and control and maintain GI tract and renal blood flow to correct hypovolaemia (Tonkonog and Bliskslager 2004, McCornico et al. 2008, Cook and Blilkslager 2015). The side effects of NSAIDs in the GI tract include GI ulceration, right dorsal colitis, and diarrhoea in foals (McCornico et al. 2008, Cook and Blilkslager 2015). On the other hand, flunixin meglumine (FM) supports the recovery of the colonic mucosa in the ischemic segment of the large colon (Martyszewski et al. 2009). Although several studies have been conducted to investigate the adverse effects of NSAIDs on horses, only a few reports have described their effects on the equine urinary system, probably because they happen rarely under recommended doses and are associated mainly with hypovolaemia. Horses have the potential to develop renal papillary necrosis if they are treated with overdoses of phenylbutazone (PHZ) or, to a lesser extent, FM (McAllister et al. 1993), or when they are treated with PHZ and are deprived of water (Gunson and Soma 1983). Side effects that have been reported after using metamizole (M2Z) in human and veterinary medicine include mild GI tract disorders, aplastic anaemia, skin allergy and renal dysfunction (Jastrebeck et al. 2014, Bents 2015). Diagnostically, urinary parameters, such as urinary gamma glutamyl transferase and urinary creatinine ratio (U-GGT-U-Cr) and fractional excretion of sodium (FE-Na), can be used to detect the effect of PHZ on the kidney function (El-Ashker et al. 2012). The urinary tract is systemically and ascendingly exposed to the pathogens, however, urinary mucus and Immunoglobulin A (IgA) play an important role in protecting the urinary tract. The mucus layer of mucous membranes is a sufficient defence mechanism to prevent adhesion of bacteria to the epithelial cells and clear them from the system (Hansson 2012). Previous studies have shown that urinary tract infections (UTIs) in horses are associated with lesions which interfere with urine
flow or can cause urinary mucosal damage (Saule et al. 2005, Saquinas and Britton 2013). Recent reports have indicated that PHZ can affect the protective mechanisms of the urinary system: while one report has shown that long-term use of PHZ can cause ulcerative cystitis in horses, presumably by reducing prostaglandin production (Alderman et al. 2011), another study has reported that using PHZ for seven days has no effect on COX-1 and COX-2 expression in bladder mucosa (Nieto et al. 2012). In a study performed on children's urine, it has been found that PHZ significantly increases urinary concentrations of TUTs (Deo and Vaidya 2004). As NSAIDs are used commonly in equine practices and little is known about their effects on the urinary system, the present study was to investigate the effects of PHZ on different equine urinary parameters, mucus and IgA concentrations in horses.

Materials and Methods

Animals and Study Design

Thirty horses, 19 females, 1 male and 10 geldings, of different breeds (21 Warmbloods, 3 Arabians, 2 ponies, 1 quarter horse, 1 Frisian, 1 Icelandic horse and 1 draft horse), ranging between 2 and 24 years (median 18.5 years) in age, and between 150 and 681 kg (median 524.5 kg) in weight, were used as a control group to determine the normal haematochemical, biochemical and urinary values, and normal urinary mucus and IgA concentrations. These horses were included in this group based on their normal clinical and clinicopathological examinations. Some of these horses were teaching herd horses. The remaining horses were client-owned and presented to the clinic for soundness examinations. They were kept in straw-bedded boxes, and were provided with hay (1.5 kg/100 kg BW/d) and concentrate (0.2 kg/100 kg BW/d) twice daily and water ad libitum. The sick horses were admitted to the Department of Large Animal Medicine, Faculty of Veterinary Medicine, University of Leipzig, Germany, between April 2015 and July 2016. All horses were diagnosed and treated. In addition to the control group, 20 horses were treated with NSAID, and, depending on the type of NSAIDs used, were assigned to one of the following groups: Horses with left dorsal colon displacement were treated with FM in group 1 (n = 9 horses). According to the intravascular volume deficit colic horses were rehydrated with lactated ringer's solution within 30 to 60 minutes. The calculation of the intravascular volume deficit was done based on the formula: (patient's PCV in % minus normal PCV) divided by normal PCV in %) multiplied with 100 = percentage of intravascular volume deficit. The continuous fluid treatment was done with 50 to 75 mL/kg BW/d i.v. using lactated ringer's solution and feed and water were withheld. All these horses had normal rectal findings at the end of the second day of treatment. After that time, horses had water ad libitum, were fed a small amount of hay three times a day and received no more fluid treatment, but still FM (q12h) treatment until day 3 after admission. Two horses of this group were excluded because they were treated with i.v. fluids during the third day. Horses with left ventral colon impaction were treated with AZ and assigned to group 2 (n = 6 horses). These horses were treated with isotonic sodium sulphate (1.8%; 20 mL/kg BW/d) orally via nasogastric tube and the impaction was solved within 48 h. During treatment, the horses had water ad libitum and the feed was withheld. After resolving, the horses received a small amount of hay four times a day and were still treated with MZ until day three. Group 3 included horses with lameness grade 1/2 and were administered PHZ for three to five days (n = 7 horses). All the patients were treated with either FM (1.1 mg/kg BW, i.v., Q12h, for 3 days), Fenadyne®, MSD, Germany; MZ (40 mg/kg BW, i.v., Q12h, for 3 days), Metapyrin®, Serumwerk Bernburg AG, Germany) or PHZ (4.4 mg/kg BW, p.o., Q24h, for 3 to 5 days; Butsan®, Selectavet Dr. Otto Fischer GmbH, Germany). All horses of all groups had no cardiovascular and/or renal disorders. The colic horses and lame horses did not receive any treatment before admission at the university animal hospital. The Animal Welfare Commission gave the ethical approval (W 06/16).

Blood and Urine Samples

Both blood and urine samples were collected from the horses of group 1 and 2 at the same time on the third day of treatment, and from the horses of group 3 on day three to five of treatment. Samples from the control group were collected on day two of hospitalisation. All samples were collected in the morning. Blood and serum samples were collected in 4 mL ethylenediaminetetraacetic acid and 10 mL plain tubes, respectively, from the jugular vein. Complete blood count was performed immediately on blood samples using the haematological analyser ADVIA 120 (Siemens Healthcare Diagnostics, Dreieich, Germany). Serum samples were centrifuged at 1100 g for 10 minutes at 4 °C, then stored at -20 °C until further analysis. The whole amount of urine was collected from female patients using a 50 cm long plastic urethral catheter as described by Schaefer and Osiris (2014). The urine samples of stallions and geldings were collected using a collecting bag, which was placed on the horse's waist until the horse urinated, then transferred into collecting tube immediately.

Renal Function

In this experiment, analyses of serum electrolytes (Na, Cl, K, Mg, Ca, IP), blood urea nitrogen (BUN), serum creatinine (S-Cr), serum glucose (S-Glu) and serum total protein (S-Prot) were performed. Urinary pH was measured with a pH electrode and specific gravity was measured using a urinometer for each urine sample within 30 minutes of collection. The rest of the urine samples were centrifuged at 500 for 5 min at 4 °C and stored at -20 °C until further analysis of urinary electrolytes (Na, Cl, K, and Mg), urinary urea nitrogen (U-UN), urinary creatinine (U-Cr), urinary glucose (U-Glu), urinary protein (U-Pro) and urinary gamma glutamyl transferase (U-GGT). In order to obtain an accurate measurement of the Ca and Pi in the urine, one aliquot of each urine sample was mixed with acetic acid for 30 min to solubilize calcium carbonate, calcium phosphate and calcium oxalate crystals (Schöff 2010) before the Ca and IP concentrations were measured. Both serum and urinary parameters were measured using the automatic analyser Hitachi 912 (Roche Diagnostics, GmbH, Mannheim, Germany). The reagents for the automatic analy-
ser were purchased from Roche Diagnostics and Randox (Randox Ltd., Krefeld, Germany). All of the haematological and serum and urinary biochemical values were expressed using the International System of Units; furthermore, the urinary GGT-U-Cr ratio in IU/mm mol, U-Cr:S-Cr ratio in μmol/μmol, U-Pro:U-Cr ratio in mg/mmol, UUN:BUN ratio in mmol/mmol, and fractional excretions (FE) of No, Ca, K, Mg, inorganic Phosphate (Pi) and Cr were calculated in percentage. The FE of electrolytes was calculated using the following equation:

$$FE = \frac{S-Cr \times \text{electrolyte concentration in urine}}{U-Cr \times \text{electrolyte concentration in serum}} \times 100$$

**Urinary Mucus and IgA Measurements**

The urinary mucous concentration was measured using a modified version of the method of Björnsson (1993, 1998). The dye solution was prepared by dissolving 900 mg Alcian Blue GP® in 100 ml of a solution that consisted of 30 ml sulfuric acid 0.5% and 5ml of 8 M urea solution. An amount of 50μl 8 M urea solution and 750μl of dye solution were pipetted into 50μl of each urine sample then incubated for 16h in a refrigerator at 4°C. Thereafter, the sample was centrifugated (13,000×g for 15 min) and the supernatant was discarded. The remaining precipitate was washed with a solution consisting of 0.5 M MgCl₂ solution in 40% dimethyl sulfoxide, centrifuged again (13,000×g for 15 min) and the supernatant wash solution was discarded; the washing and centrifugation were repeated three times. The urine mucous-dye complex was dissolved in 500μl of a solution consisting of 4 M urea solution/n-propanol (2:1; v/v). The intensity of the resulting blue solution was measured by an ultraviolet-visible photometer (Beckman 640-DU®) at 620 nm. In the absence of equine mucin as a reference material, porcine mucin type II® was used as a standard for calibration and control (Kölker et al. 2010). An amount of 200μl of urine and 200μl of 2 M sodium hydroxide solution were treated for 4h at 95°C for the negative control. After cooling, the mixtures were neutralized with 100μl of sulfuric acid (10%), then the remaining urinary mucous concentrations were measured by the same method described above. All urine samples and positive and negative controls were measured in triplicate, and the mean values were used for statistical analysis. Measurement of urinary IgA was performed using horse IgA ELSA, as described by the manufacturer, with slight modification. Because the total urinary protein concentration was very low and the optimal IgA concentration was needed, one of the control group samples and another from a horse diagnosed with haemorrhagic cystitis were used in three different concentrations (undiluted, 1:10, and 1:100 using distilled water). Consequently, urine samples of the control group were used in an undiluted form, while samples from horses with suspected urinary tract inflammatory process were diluted at a ratio of 1:10. Briefly, the calibrators, diluted and undiluted samples, were added to duplicate wells in horse IgA-coated microplates, and incubated for 30 min at 22°C; after aspirating the contents of the wells, the washing solution was used in percentage. The FE of electrolytes was calculated using the following equation:

$$FE = \frac{S-Cr \times \text{electrolyte concentration in urine}}{U-Cr \times \text{electrolyte concentration in serum}} \times 100$$

**Statistical Analysis**

Statistical analyses of the data were carried out using the statistical software programme (SPSS 22, International Business Machines (IBM) cooperation, New York, USA). Data were examined for normal distribution using the Shapiro-Wilk test, which revealed that the data were not normally distributed, and their descriptive statistics were described as median, 1st and 3rd quartile, and because of urine pH values of some horses were under the detection limit, the minimum and maximum of FE were used. Differences between the control and groups tested were tested using the Kruskal-Wallis one-way analysis of variance (ANOVA) test and Dunn’s method. Significance was set at P<0.05.

**Results**

**Renal Function**

A total of 50 horses were included in this experiment, of which 20 horses were treated with FM, MZ or PHZ. Of these 20 horses, 10 were females, 9 were geldings and 1 was male; their ages ranged between 7 and 24 years (median 21 years). They were of different breeds including 11 Warmbloods, 2 Friesian, 2 Arabians, 1 Haflinger, 1 Icelandic horse, 1 pony, 1 Lippizaner and 1 Holsteiner horse. There were no differences between all groups and control group regarding age and gender. There was a significant difference

| Table 1 | Clinical and haematological findings of the healthy horses (control group) and groups treated (group 1: horses treated with Flumixin meglumine for three days; group 2: horses treated with mequitazone for three days; group 3: horses treated with phenylbutazone for three to five days). The results are described as median, (1st to 3rd quartile). Temp., rectal temperature; RBCs, red blood corpuscles; PCV, packed cell volume; WBCs, white blood cells; n, number of horses in each group. *Significantly different values compared with those of the control group. |
|---------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| Variables | Normal range of all breeds | Control group (n = 30) | Group 1 (n = 7) | Flumixin meglumine | Group 2 (n = 6) | Group 3 (n = 7) | Phenybutazone |
| Temp. (°C) | 37.5 – 38.0 | 37.6 (37.4 – 37.9) | 38 (37.6 – 38.9)* | 38.4 (37.2 – 38.9) | 37.5 (37.3 – 37.7) | |
| RBCs (x 10¹²/L) | 5.1 – 11.9 (1) | 7.4 (7.0 – 8.1) | 7.1 (6.5 – 7.2) | 6.2 (5.9 – 7.2)* | 6.67 (6.23 – 7.24) | |
| PCV (L/L) | 0.22 – 0.39 (1) | 0.34 (0.31 – 0.36) | 0.31 (0.29 – 0.31)* | 0.31 (0.29 – 0.33)* | 0.32 (0.28 – 0.32) | |
| WBCs (x 10¹⁷/L) | 4.4 – 16.5 (1) | 7.1 (6.0 – 9.7) | 7.9 (6.4 – 9.7) | 8.6 (6.9 – 10.3) | 7 (6.1 – 9.3) | |

(1) Kölker et al. 2014

*Significantly different values compared with those of the control group.
### Table 2
Serum biochemical parameters of the healthy horses (control group) and groups treated (group 1: horses treated with flunixin meglumine for three days; group 2: horses treated with metamizole for three days; group 3: horses treated with phenylbutazone for three to five days). The results are described as median, (1st to 3rd quantile). Pi: inorganic phosphorus; S-Cr: serum creatinine; U-N: blood urea nitrogen; *Significantly different values compared with those of control group.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normal range of all breeds</th>
<th>Control group (n = 30)</th>
<th>Group 1 (n = 7)</th>
<th>Group 2 (n = 6)</th>
<th>Group 3 (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na (mmol/L)</td>
<td>134 – 135</td>
<td>138 (136 – 140)</td>
<td>139 (137 – 139)</td>
<td>139 (137 – 141)</td>
<td>139 (138 – 140)</td>
</tr>
<tr>
<td>Cl (mmol/L)</td>
<td>88.8 – 114.6</td>
<td>100 (96.5 – 101.2)</td>
<td>99.6 (95.2 – 101.6)</td>
<td>98.9 (95.1 – 103.9)</td>
<td>100.7 (97.1 – 103.6)</td>
</tr>
<tr>
<td>K (mmol/L)</td>
<td>2.0 – 4.8</td>
<td>3.7 (3.4 – 4.2)</td>
<td>3.8 (3.6 – 3.98)</td>
<td>3.6 (3.2 – 3.7)</td>
<td>3.93 (3.4 – 4.2)</td>
</tr>
<tr>
<td>Ca (mmol/L)</td>
<td>2.62 – 3.23</td>
<td>3.1 (2.8 – 3.2)</td>
<td>2.99 (2.83 – 3.10)</td>
<td>3.1 (2.7 – 3.2)</td>
<td>3.1 (2.9 – 3.4)</td>
</tr>
<tr>
<td>Mg (mmol/L)</td>
<td>0.25 – 0.33</td>
<td>0.27 (0.24 – 0.30)</td>
<td>0.29 (0.26 – 0.32)</td>
<td>0.31 (0.27 – 0.35)</td>
<td>0.30 (0.26 – 0.35)</td>
</tr>
<tr>
<td>Pi (mmol/L)</td>
<td>0.42 – 3.03</td>
<td>0.47 (0.43 – 1.10)</td>
<td>0.88 (0.68 – 1.01)</td>
<td>0.82 (0.67 – 0.80)</td>
<td>0.8 (0.7 – 1.5)</td>
</tr>
<tr>
<td>S-Cr (mmol/L)</td>
<td>51.0 – 156.4</td>
<td>106 (87 – 115)</td>
<td>115 (103 – 133)</td>
<td>99.9 (93 – 128)</td>
<td>112 (98 – 127)</td>
</tr>
<tr>
<td>U-N (mmol/mol)</td>
<td>1.22 – 8.95</td>
<td>4.4 (4.0 – 5.8)</td>
<td>4.8 (5.2 – 5.4)</td>
<td>4.5 (3.7 – 5.35)</td>
<td>4.75 (4.6 – 5.4)</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>2.18 – 9.17</td>
<td>5.9 (5.1 – 6.5)</td>
<td>5.7 (4.9 – 6.99)</td>
<td>5.2 (4.5 – 6.3)</td>
<td>5.14 (4.50 – 5.43)</td>
</tr>
<tr>
<td>Total protein</td>
<td>48.0 – 81.4</td>
<td>64.3 (61.1 – 70.0)</td>
<td>63.1 (44.3 – 70.2)</td>
<td>65.95 (54.9 – 70.0)</td>
<td>60.4 (59.4 – 66.8)</td>
</tr>
</tbody>
</table>

(1) Koller et al. 2014

### Table 3
Urinary parameters of healthy horses (control group) and groups treated (group 1: horses treated with flunixin meglumine for three days; group 2: horses treated with metamizole for three days; group 3: horses treated with phenylbutazone for three to five days). The results are described as median, (1st to 3rd quantile). n, number of horses in each group; U-GGT, urinary gamma glutamyl transferase; FECl, fractional excretion of sodium; FEPo4, fractional excretion of phosphate; FEPa, fractional excretion of potassium; FEPa3, fractional excretion of calcium; FEPa, fractional excretion of inorganic phosphate; FEMP, fractional excretion of magnesium; U-GGT-U-Cr, urinary gamma glutamyl transferase/urinary creatinine ratio; U-Cr35, urinary creatinine/serum creatinine ratio; U-Pro-U-Cr, urinary protein/urinary creatinine ratio; U-N:UN, urinary urea nitrogen/serum blood urea nitrogen ratio; FECl expressed as minimum and maximum values as some IP in some horses was below the detectable limit; NA, not available; Urinary GGT in some horses of group 1 was below the detectable limit. *Significantly different values compared with those of control group.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Normal range of all breeds</th>
<th>Control group (n = 30)</th>
<th>Group 1 (n = 7)</th>
<th>Group 2 (n = 6)</th>
<th>Group 3 (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific gravity</td>
<td>1.030 – 1.050</td>
<td>1.035</td>
<td>1.026 (1.028 – 1.043)</td>
<td>1.023 (1.014 – 1.042)</td>
<td>1.038 (1.030 – 1.054)</td>
</tr>
<tr>
<td>pH</td>
<td>7.6 – 8.4</td>
<td>7.65</td>
<td>7.94 (7.23 – 8.00)</td>
<td>7.3 (7.10 – 7.59)</td>
<td>7.53 (6.92 – 7.90)</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>not available</td>
<td>0.5</td>
<td>0.37 (0.33 – 0.60)</td>
<td>0.37 (0.17 – 0.42)</td>
<td>0.68 (0.61 – 0.80)</td>
</tr>
<tr>
<td>Protein (g/L)</td>
<td>&lt; 0.4</td>
<td>0.07</td>
<td>0.2 (0.05 – 0.11)</td>
<td>0.18 (0.08 – 0.57)</td>
<td>0.18 (0.035 – 0.350)</td>
</tr>
<tr>
<td>FECl (%)</td>
<td>0.032 – 0.520</td>
<td>0.69</td>
<td>0.18 (0.035 – 0.072)</td>
<td>0.39 (0.039 – 0.320)</td>
<td>0.39 (0.029 – 0.036)</td>
</tr>
<tr>
<td>FEPa (%)</td>
<td>0.59 – 1.86</td>
<td>0.76</td>
<td>0.9 (0.47 – 1.02)</td>
<td>0.92 (0.52 – 0.99)</td>
<td>0.31 (0.18 – 0.58)</td>
</tr>
<tr>
<td>FEMP (%)</td>
<td>23.3 – 48.1</td>
<td>35.9</td>
<td>35.6 (26.63 – 48.10)</td>
<td>46.29 (38.80 – 65.32)</td>
<td>19.61 (11.42 – 28.26)</td>
</tr>
<tr>
<td>FEPO4 (%)</td>
<td>0.672</td>
<td>5.37</td>
<td>6.5 (2.46 – 8.60)</td>
<td>6.5 (3.32 – 12.60)</td>
<td>4.6 (3.39 – 5.05)</td>
</tr>
<tr>
<td>FEPro (%)</td>
<td>0.3</td>
<td>0.044</td>
<td>0.054 (0.324)</td>
<td>0.054 (0.324)</td>
<td>0.066 (0.414)</td>
</tr>
<tr>
<td>FEUN (%)</td>
<td>20.8 – 43.1</td>
<td>11.39</td>
<td>15.31 (6.79 – 20.20)</td>
<td>15.31 (12.80 – 23.08)</td>
<td>16.27 (10.94 – 22.60)</td>
</tr>
<tr>
<td>U-GGT-U-Cr</td>
<td>&lt; 2.62</td>
<td>0.073</td>
<td>NA</td>
<td>0.038 (0.009 – 0.290)</td>
<td>0.106 (0.074 – 1.86)</td>
</tr>
<tr>
<td>U-S-Cr</td>
<td>2 – 344</td>
<td>215.7</td>
<td>153.3 (164.90 – 314.97)</td>
<td>108.49 (79.06 – 134.79)</td>
<td>145.7 (103.99 – 313.97)</td>
</tr>
<tr>
<td>U-Pro-U-Cr</td>
<td>not available</td>
<td>0.003</td>
<td>0.008 (0.002 – 0.006)</td>
<td>0.008 (0.004 – 0.070)</td>
<td>0.004 (0.009 – 0.028)</td>
</tr>
<tr>
<td>U-N:UN</td>
<td>20 – 124</td>
<td>61.05</td>
<td>45.43 (45.5 – 79.9)</td>
<td>45.43 (26.43 – 62.70)</td>
<td>36.4 (31.3 – 48.6)</td>
</tr>
</tbody>
</table>


15
Results

(P < 0.029) in the rectal temperatures of the horses in group 1 in comparison with those of the control group (Tab. 1). Haematological values, such as red blood cell (RBC) and white blood cell (WBC) counts and packed cell volume (PCV) of all groups were within normal ranges and are listed in Table 1 (Köfler et al. 2014). The serum biochemical values are summarized in Table 2. Most of the serum biochemical values of the groups treated did not differ significantly from those of the control group. Serum Mg was significantly lower in group 2 (P < 0.045) compared with the control group. Urinary parameters of the control and groups treated are listed in Table 3. Urine specific gravity decreased significantly in the horses of group 2 (P < 0.002) compared with the control group. Urinary protein concentration was significantly higher in group 1 (P < 0.037) and group 2 (P < 0.021) compared with the control group. The FENa was significantly higher in group 1 (P < 0.018) and group 3 (P < 0.005), and significantly lower in group 2 (P < 0.017) in comparison with the control group. The FECI was significantly lower in group 2 (P < 0.02) when compared with the control group. Only group 2 showed a significant decrease in the FE of potassium of P < 0.008 compared with the control group. Significant increases in the FE of calcium and magnesium were observed in group 3 (P < 0.02), (P < 0.033) respectively, when compared with the control group. The FEiP of the horses in all groups treated were within the normal range, as presented in Table 3. The U-GGT:U-Cr ratio was significantly higher in group 3 (P < 0.001) compared with the control group. The U-Pro:U-Cr ratio was significantly higher in group 1 (P < 0.007) and group 2 (P < 0.001) than the control group. The U-Cr:S-Cr ratios of group 1 (P < 0.056) and 2 (P < 0.001) showed significant decreases compared with the control group. The UUN:BUN ratio was significantly lower in group 2 (P < 0.03) than the control group. Because the urinary phosphate concentration in some horses in all groups were below the detectable limit, in addition to U-GGT concentrations of some horses in group 1, their statistics could not be calculated.

Urinary Mucus and IgA Concentrations

Both urinary mucus and IgA values of the control group and groups treated were summarized as median and 1st and 3rd quartile in Table 4 and 5, respectively. No influence of the method of urine collection on the mucus concentration was found. There was a significant difference in the urinary mucus:U-Cr ratio of the horses in group 3 (P < 0.005) compared with those in the control group, and no significant differences between the other groups and the control group or among the groups have been detected (Tab. 4). The urinary IgA concentration was significantly higher in group 2 (P < 0.022). Groups 2 and 3 showed a significantly increased IgA:U-Cr ratio (P < 0.007 and P < 0.014, respectively) compared with the control group (Tab. 5). No significant differences were detected among the groups treated regarding the urinary IgA concentration and the IgA:U-Cr ratio.

Discussion

The current study describes renal function parameters in horses treated for three to five days with NSAIDs for gastrointestinal (FM, MZ) or orthopedic (PHZ) pain. Our results on equine kidney functions using FM, MZ and PHZ were compared with findings in the literature (MacAllister et al. 1993, El-Ashkier et al. 2012). The limitation of this study is the samples were collected on days 3–5 of treatment, no samplings were performed before treatment, as these horses were patients brought to the clinic to be treated for their gastrointestinal and orthopedic conditions. Hypoproteinaemia is a sign of acute renal disorders, although not all horses with acute renal diseases develop hypoproteinaemia, and several reports indicated that hyperkalaemia is usually associated with renal disorder (Gneer 2007, Toribio 2007). Because the patients in groups 1 and 2 were treated with lactated ringer’s solution, the serum Na and K were within normal range in these groups, what indicates that these NSAIDs may have no effects on serum Na and K, despite their effects on FENa and FEK. The NSAIDs used did not influence the reabsorption of inorganic phosphate in the proximal tubules, because the FEPI were not increased in all groups compared to control group. Some horses of group 2 developed a mild to moderate form of hypoproteinaemia in comparison with the control group, but still within the reference range, which, in combination with a mildly increased FENa and reduced U-Cr:S-Cr ratio, suggests that using MZ may slightly reduce the reabsorption of

Table 4  Urinary mucus concentrations and mucus:U-Cr ratios of the healthy horses (control group) and groups treated (group 1: horses treated with flunixin meglumine for three days; group 2: horses treated with metamizole for three days; group 3: horses treated with phenylbutazone for three to five days). The results are described as median, (1st to 3rd quartile), n, number of horses in each group. *Significantly different values compared to those of control group.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group (n = 30)</th>
<th>Group 1 (n = 7) Flunixin meglumine</th>
<th>Group 2 (n = 6) Metamizole</th>
<th>Group 3 (n = 7) Phenylbutazone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucus (g/l)</td>
<td>1.30 (0.84 – 1.56)</td>
<td>0.77 (0.46 – 1.39)</td>
<td>0.60 (0.41 – 1.69)</td>
<td>1.56 (0.73 – 2.54)</td>
</tr>
<tr>
<td>Mucus:U-Cr (g/mmol)</td>
<td>0.05 (0.04 – 0.06)</td>
<td>0.05 (0.04 – 0.06)</td>
<td>0.06 (0.03 – 0.14)</td>
<td>0.07 (0.07 – 0.09)*</td>
</tr>
</tbody>
</table>

Table 5  Urinary IgA concentrations and IgA:U-Cr ratios of the healthy horses (control group) and groups treated (group 1: horses treated with flunixin meglumine for three days; group 2: horses treated with metamizole for three days; group 3: horses treated with phenylbutazone for three to five days). The results are described as median, (1st to 3rd quartile), n, number of horses in each group. *Significantly different values compared to those of control group.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group (n = 30)</th>
<th>Group 1 (n = 7) Flunixin meglumine</th>
<th>Group 2 (n = 6) Metamizole</th>
<th>Group 3 (n = 7) Phenylbutazone</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA (µg/l)</td>
<td>139.8 (37.4 – 371.7)</td>
<td>515.8 (244.8 – 576.3)*</td>
<td>381.5 (107.6 – 972.5)</td>
<td>49.5 (8.0 – 481.8)</td>
</tr>
<tr>
<td>IgA:U-Cr (µg/mmol)</td>
<td>5.3 (1.8 – 16.7)</td>
<td>26.0 (17.9 – 62.0)*</td>
<td>27.0 (8.9 – 77.6)*</td>
<td>4.6 (0.2 – 29.9)</td>
</tr>
</tbody>
</table>
Mg at the thick ascending loop of Henle, although FEmg in this group is not significantly different from the control group. It has been reported that 50 to 70% of the Mg in horses is reabsorbed in the thick ascending loop of Henle (Sorribio 2007). Normal S-Cr, glucose, total protein and BUN concentrations of all groups (compared to the control group) indicate that renal side effects caused by FM, MZ and PHZ cannot be detected using these serum biochemical parameters on the third or until the fifth day of administrations. A previous study, in which PHZ was used at a dose of 4.4 mg/kg, i.v., Q8H, for 12 days, reported that using PHZ for 8–days caused significant hyperproteinenaemia and hyperbilirubinaemia, and further use of PHZ up to 12 days caused even more reduction in serum total protein and albumin concentrations, whereas FM had no effect on equine serum protein concentrations (MacAllister et al. 1993). The difference from our study is that the PHZ dose was used three times a day and length of treatment was longer in the latter study, which suggests that prolonged and more frequent use of PHZ can lead to protein-losing nephropathies. Urinary specific gravity is affected by the concentration of the solutes in the urine. Decreased concentrations of UU/N, U-Cr, K, Ca and Cl in group 2 caused a urine specific gravity less than 1.035 (control group), however, MZ does not cause polyuria, but the horses with colic treated with laxatives could have more water intake after resolving the impaction (Spalliek et al. 2011). Therefore, group 2 showed significantly decreased urinary FENa, which is probably a renal compensatory mechanism. The FECI and FEK were significantly decreased in group 2, which could be influenced by the laxative treatment with sodium sulphate (1.8%) (Spalliek et al. 2011). The loop of Henle controls urine concentration via a countercurrent multiplication mechanism, which depends mainly on concentrations of urea and sodium salts in the renal medullary interstitial fluids (Sadowski and Dobrakowski 2000, Schott 2010). In the present study, both U-Cr and UU/N concentrations were significantly lower in both groups 1 and 2 than in the control group. This could be partially because of the slightly low BUN concentrations of these two groups, which could be due, in turn, to the i.v. fluid treatment (group 1) and more water intake (group 2) rather than loop of Henle dysfunction. Using PHZ did not affect the UU/N concentration, however, it caused a significant increase of FECa and FEMg in comparison with the control group, which suggests that using PHZ in horses with orthopaedic pain can affect the reabsorption of Ca and Mg at the thick ascending loop of Henle. Previous studies pointed to renal crest necrosis as a side effect of using PHZ, since the PHZ can cause inadequate blood supply in the renal medulla in water-deprived horses (Gunson and Soma, 1983, MacAllister 1993). However, the difference between the latter studies and our study is that, unlike these two studies, the horses in group 3 of our study were hydrated (as their PCV showed), and these horses were administered the recommended dose of PHZ. The dosage of PHZ used caused a high-grade increase of the U-GGT/U-Cr ratio in group 3 in comparison with the control group, but it is still within the normal range and does not indicate a damaging effect on brush border cells of the renal proximal tubular epithelium. Only a high U-Pro/U-Cr ratio can be used to detect glomerular or tubular dysfunction. The U-Pro/U-Cr ratio of group 1 and 2 were significantly increased compared with the control group, which suggests a mild tubular dysfunction. Our results suggest that the concentration of the 3rd quartile of the urine protein was higher than the normal range only in group 1 (FM). In an experimental study to investigate the effect of daily FM administration for five days on clinical and clinicopathological parameters in foals, the FM did not affect the PCV in the foals treated (Carrick et al. 1989). One of the side effects of PHZ in horses is anorexia; as the horses treated with PHZ become anorectic, they drink inadequate amounts of water, which causes the urine to be concentrated, which appeared in the specific gravity of the urine in this group compared with the control group. This suggestion is consistent with another report that PHZ can cause anorexia in the horses treated and subsequent progressive hypercreatininaemia (MacKay et al. 1983). However, horses treated with PHZ in this study were not anorectic and had no hypercreatininaemia. Horses in groups 1 and 2 showed no significantly decreased medians of urinary mucus concentrations, however, their mucus:U-Cr ratios were within the same range compared with the control group. Horse’s urine is rich in mucus, mainly to prevent the formation of urinary stones and subsequent UTIs. A significant increase in the IgA:U-Cr ratios of the horses in groups 1 and 2 has been observed in this study. Mucosal IgA is secreted by B lymphocytes, then transported through epithelial cells into the organ’s lumen due to mucosal stimulation (Tezuka et al., 2007, Lewis et al. 2010). The increased urinary IgA concentrations in both groups 1 and 2 could be caused by the influence of FM or MZ on the function of the proximal tubules.

Conclusion

The current study illustrates effects of commonly used NSAIDs on kidney functions, urinary mucus, and IgA secretions in horses. Parameters such as U-Pro:U-Cr and U-GGT:U-Cr ratios and FEMg, in combination with other clinical and clinicopathological values, can be used to detect dysfunction caused by FM, MZ and PHZ in the renal proximal tubules and the loop of Henle in equine kidneys. The renal pelvis can increase urinary mucus secretion, presumably as a preventive mechanism against the necrotic effect of PHZ in the adjacent renal papillae. Different possibilities could be attributed to the elevated urinary IgA secretion, due to glomerular and tubular inflammatory reaction or leaked through damaged renal glomeruli, therefore, further investigations regarding urinary immune responses are required.

Acknowledgment

The authors would like to thank Ms. Annekathrin Ruhtland, Ms. Carola Näther and Ms. Julia Schwippel, technicians at the Clinical Pathological Laboratory, Department of Large Animal Medicine, Faculty of Veterinary Medicine, Leipzig University, for their technical assistance.

Manufacturers’ addresses

1 Co. Sigma-Aldrich, Taufkirchen, Bavaria, Germany.
2 Co. Beckman-Coulter, Krefeld, Nordrhein-Westfalen, Germany.
3 Kamiya Biochemical Company, Seattle, Washington, USA.
4 Thermo Fisher Scientific, Erlangen, Bavaria, Germany.
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Hollmayer T. (1999) Quantitative and qualitative urinary protein excretion in healthy and sick horses. Doctoral Thesis, Faculty of Veterinary Medicine, University of Leipzig


Erweiterte Zusammenfassung

Auszüge aus verschiedenen Publikationen, die die Auswirkungen auf die Immunglobulin A (IgA)-Konzentration erläutern:


4. Discussion

The current study describes the effects of using flunixin meglumine (FM), metamizole (MZ) (for gastrointestinal pain) or phenylbutazone (PHZ) (for orthopedic pain) on renal function parameters in horses. Our results on equine kidney functions using FM, MZ and PHZ were compared with findings in the literature (MACALLISTER et al. 1993; EL-ASHKER et al. 2012). The limitation of this study is the samples were collected on days 3-5 of treatment, no samplings were performed before treatment, as these horses were patients brought to the clinic to be treated for their gastrointestinal and orthopedic conditions. Because the patients in groups 1 and 2 were treated with lactated ringer’s solution, the serum Na and K were within normal range in these groups, which indicate that these NSAIDs may have no effects on serum Na and K, despite their effects on $F_{E_{Na}}$ and $F_{E_{K}}$. The NSAIDs used did not influence the reabsorption of inorganic phosphate in the proximal tubules, as the $F_{E_{ip}}$ values were not increased in all groups compared to control group. Some horses of group 2 developed a mild to moderate form of hypomagnesemia in comparison with the control group, but still within the reference range, which, in combination with a mildly increased $F_{E_{Mg}}$ and reduced U-Cr:S-Cr ratio, suggests that using MZ may slightly reduce the reabsorption of Mg at the thick ascending loop of Henle, although $F_{E_{Mg}}$ in this group is not significantly different from the control group. It has been reported that 50 to 70 % of the Mg in horses is reabsorbed in the thick ascending loop of Henle (TORIBIO 2007). Normal S-Cr, glucose, total protein and BUN concentrations of all groups (compared to the control group) indicate that renal side effects caused by FM, MZ and PHZ cannot be detected using these serum biochemical parameters on the third or until the fifth day of administrations. A previous study, in which PHZ was used at a dose of 4.4 mg/kg, i.v., q8h, for 12 days, reported that using PHZ for 8 days caused significant hypoproteinemia and hypoalbuminemia, and further use of PHZ up to 12 days caused even more reduction in serum total protein and albumin concentrations, whereas FM had no effects on equine serum protein concentrations (MACALLISTER et al. 1993). The difference from our study is that the PHZ dose was used three times a day and length of treatment was longer in the latter study, which suggests that prolonged and more frequent usage of PHZ can lead to protein-losing nephropathy. Urinary specific gravity is affected by the concentration of the solutes in the urine. Decreased concentrations of UUN, U-Cr, K, Ca and Cl in group 2 caused a urine specific gravity less than 1.035 (control group), however, MZ does not cause polyuria, but the horses with colic treated with laxatives could have more water intake after resolving the
impaction (SPALLEK et al. 2011). Therefore, group 2 showed significantly decreased urinary FE$_{Na}$, which is probably a renal compensatory mechanism. The FE$_{Cl}$ and FE$_{K}$ were significantly decreased in group 2, which could be influenced by the laxative treatment with sodium sulphate (1.8 %) (SPALLEK et al. 2011). The loop of Henle controls urine concentration via a countercurrent multiplication mechanism, which depends mainly on concentrations of urea and sodium salts in the renal medullary interstitial fluids (SADOWSKI and DOBROWOLSKI 2003; SCHOTT 2010). In the present study, both U-Cr and UUN concentrations were significantly lower in both groups 1 and 2 than in the control group. This could be partially because of the slightly low BUN concentrations of these two groups, which could be due, in turn, to the i.v. fluid treatment (group 1) and more water intake (group 2) rather than loop of Henle dysfunction. Using PHZ did not affect the UUN concentration, however, it caused a significant increase of FE$_{Ca}$ and FE$_{Mg}$ in comparison with the control group, which suggests that using of PHZ in horses with orthopedic pain can affect the reabsorption of Ca and Mg at the thick ascending loop of Henle. Previous studies pointed to renal crest necrosis as a side effect of using PHZ, since the PHZ can cause inadequate blood supply in the renal medulla in water-deprived horses (GUNSON and SOMA 1983; MACALLISTER 1993). However, the difference between the latter studies and our study is that, unlike these two studies, the horses in group 3 of our study were hydrated (as their PCV showed), and these horses were administered the recommended dose of PHZ. The dosage of PHZ used caused a high-grade increase of the U-GGT:U-Cr ratio in group 3 in comparison with the control group, but it is still within the normal range and does not indicate a damaging effect on brush border cells of the renal proximal tubular epithelium. Only a high U-Pro:U-Cr ratio can be used to detect glomerular or tubular dysfunction. The U-Pro:U-Cr ratio of group 1 and 2 were significantly increased compared with the control group, which suggests a mild tubular dysfunction. Our results suggest that the concentration of the 3rd quartile of the urine protein was higher than the normal range only in group 1 (FM). In an experimental study to investigate the effect of daily FM administration for five days on clinical and clinicopathological parameters in foals, the FM did not affect the PCV in the foals treated (CARRICK et al. 1989). One of the side effects of PHZ in horses is anorexia; as the horses treated with PHZ become anorectic, they drink inadequate amounts of water, which causes the urine to be concentrated, which appeared in the specific gravity of the urine in this group compared with the control group. This suggestion is consistent with another report that PHZ can cause anorexia in the horses treated and subsequent progressive hypercreatininemia (MACKAY et al. 1983). However, horses treated with PHZ in
this study were not anorectic and had no hypercreatininemia. Horses in groups 1 and 2 showed no significantly decreased medians of urinary mucus concentrations, however, their mucus:U-Cr ratios were within the same range compared with the control group. Horse’s urine is rich in mucus, mainly to prevent the formation of urinary stones and subsequent UTIs. A significant increase in the IgA:U-Cr ratios of the horses in group 1 and 2 has been observed in this study. Mucosal IgA is secreted by B lymphocytes, then transported through epithelial cells into the organs’ lumen due to mucosal stimulation (TEZUKA et al., 2007; LEWIS et al., 2010). The increased urinary IgA concentrations in both groups 1 and 2 could be caused by the influence of FM or MZ on the function of the proximal tubules.

This study illustrates the effects of the NSAIDs used most commonly on the kidneys’ functions, and urinary mucus and IgA secretions in horses. Parameters such as U-Pro:U-Cr and U-GGT:U-Cr ratios and FE\textsubscript{Mg}, in combination with other clinical and clinicopathological values, can be used to detect dysfunction caused by FM, MZ and PHZ in the renal proximal tubules and the loop of Henle in equine kidneys. The renal pelvis can increase urinary mucus secretion, presumably as a preventive mechanism against the necrotic effect of PHZ in the adjacent renal papillae. Different possibilities could be attributed to the elevated urinary IgA secretion, due to glomerular and tubular inflammatory reaction or leaked through damaged renal glomeruli, therefore, further investigations regarding urinary immune responses are required.
5. Summary

Mohammed I. Adam

Effects of flunixin meglumine, metamizole and phenylbutazone on equine kidney functions and urinary mucus and immunoglobulin A (IgA) secretions.

Large Animal Clinic for Internal Medicine, Faculty of Veterinary Medicine, Leipzig University

Submitted in March 2018

26 pages, 1 publication, 5 tables, 82 references, 3 appendices

Keywords: kidney function, flunixin meglumine, metamizole, phenylbutazone, urinary mucus, immunoglobulin A (IgA), equine

Introduction: Nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most used drugs in equine medicine, mainly used to treat inflammation, endotoxemia, pain or fever. NSAIDs inhibit cyclooxygenases which induce to synthesize prostanoids. But NSAIDs have side effects to renal functions too.

Objectives: The current study was carried out to investigate the effects of the most common used NSAIDs on urinary parameters in horses.

Materials and Methods: Thirty healthy horses were used as a control group and 20 horses with left dorsal displacement, left ventral impaction or lameness of using either flunixin meglumine (FM), metamizole (MZ) or phenylbutazone (PHZ) have been assigned to groups 1, 2 or 3, respectively. Creatinine, urea nitrogen, glucose, protein and electrolytes were measured in serum and urine including GGT using an automatic analyzer. Fractional excretions (FE) of sodium, chloride, potassium, calcium, magnesium and inorganic phosphate, in addition to urinary protein (U-Pro):U-Cr and urinary gamma glutamyl transferase (U-GGT):U-Cr ratios were calculated. Urinary mucus and IgA concentrations were measured and their ratios to the urinary creatinine were calculated. The data were statistically analyzed using Shapiro-Wilks test, descriptive statistics, Kruskal-Wallis one-way analysis of variance and Dunn’s test. Significance was set at P ≤ 0.05.

Results: The FE_Mg was significantly higher in group 3 (P < 0.033) compared to the control group. The U-GGT:U-Cr ratio was also significantly higher in group 3 (P < 0.001) compared with the control group. The U-Pro:U-Cr ratio was significantly higher in groups 1 and 2 (P < 0.007 and P < 0.001, respectively) than in the control group. PHZ group had a significantly
Summary

increase in mucus:U-Cr ratio (P < 0.005). Significant increases were observed regarding the IgA:U-Cr ratio in groups 1 (P < 0.007) and 2 (P < 0.014).

Conclusions: Long-term use of PHZ has an influence on the renal ascending limb of the loop of Henle, and all these drugs could have effects on the proximal tubules. Phenylbutazone causes an increase in urinary mucus secretion, probably as a protective mechanism against the necrotic effect in renal pelvis of PHZ. Parameters such as U-Pro:U-Cr and U-GGT:U-Cr ratios and FE\textsubscript{Mg} are helpful in detecting these renal abnormalities.
Zusammenfassung

6. Zusammenfassung

Mohammed I. Adam

Wirkungen des Flunixin meglumin, Metamizol oder Phenylbutazon auf Nierenfunktionen, Harnmukus und Immunoglobulin-A-Konzentration

Medizinische Tierklinik, Veterinärmedizinischen Fakultät, Universität Leipzig

Eingereicht im März 2018

26 Seiten, 1 Publikation, 5 Tabellen, 82 Literaturangaben, 3 Anhänge

Schlüsselwörter: Nierenfunktion, Flunixin meglumin, Metamizol, Phenylbutazon, Harnmukus, Immunglobulin A (IgA), Pferd

Einleitung: Nichtsteroidale, antiphlogistische Medikamente (NSAIDs) werden in der Pferdemedizin sehr häufig eingesetzt, um Entzündungen, Endotoxämie, Schmerz oder Fieber zu behandeln. NSAID inhibieren Enzyme der Cyclooxygenase, die die Prostaglandinsynthese herbeiführen. Jedoch haben NSAIDs auch Nebenwirkungen auf die Nierenfunktionen.

Ziele: Die aktuelle Studie untersuchte die Wirkungen der häufig eingesetzten NSAID auf die Nierenfunktionen bei Pferden.


Ergebnisse: Die FE_Mg war signifikant höher in Gruppe 3 (P < 0,033) im Vergleich zur Kontrollgruppe. Das U-GGT: U-Cr-Verhältnis war in der Gruppe 3 (P < 0,001) im Vergleich zur Kontrollgruppe ebenfalls signifikant höher. Das U-Pro: U-Cr-Verhältnis war signifikant höher in
Zusammenfassung

den Gruppen 1 und 2 (P < 0,007 bzw. P < 0,001) als in der Kontrollgruppe. PHZ-Gruppe hatte eine signifikante Zunahme des Urinschleim:U-Cr-Verhältnisses (P < 0,005). In den Gruppen 1 (P < 0,007) und 2 (P < 0,014) wurden signifikante Zunahmen des IgA:U-Cr-Verhältnisses beobachtet.

7. References


References


References


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8. Appendixes

Publications


Conference contribution

Acknowledgment

I would like to thank my supervisor Professor Dr. Gerald Fritz Schusser, whose encouragement, guidance and support helped me to successfully complete this project.

I am also thankful to Dr. Köller for his support and helps during the laboratory work, and also all staff members of the Department of Large Animal Medicine for their support during the clinical work.

I wish to express my warm and sincere thanks to Mrs. Ruhland, Mrs. Näther, Mrs. Dögl and Mrs. Schwippel whom provided my technical support in laboratory.

I also would like to thank my parents, my siblings, and my friends for their unconditional love and support.