Acute kidney injury in horses as a consequence of treatment with suxibuzone

Zuzana Drábková[1], Karolína Bábor[1], Andrea Kopecká[1], April Jelínek[2]

University of Veterinary Sciences Brno, Faculty of Veterinary Medicine,
[1]Equine Clinic, [2]Department of Animal Genetics, Brno, Czech Republic

Received October 26, 2023
Accepted June 18, 2024

Abstract

Suxibuzone is a prodrug of the nonsteroidal anti-inflammatory drug phenylbutazone. The aim of this study was to investigate the nephrotoxicity of suxibuzone using the recommended dosage in euhydrated horses. Serum creatinine levels were used to monitor for the presence of acute kidney injury (AKI) in 11 systemically healthy horses treated orally with suxibuzone at the recommended dosage for five days. Symmetric dimethylarginine (SDMA), urine gamma glutamyl transferase/urine creatinine, fractional excretion of sodium, urine protein-to-creatinine ratio, and urine sediment were assessed as additional monitoring parameters. A significant increase in creatinine was found in treated horses (P = 0.002), 27% (3/11) of treated horses were classified as having AKI compared to 0% of controls (0/10), and a strong correlation was found between the change in creatinine and the change in SDMA in treated horses (τ = 0.645, P = 0.012). The results of this study suggest that treatment with suxibuzone at the recommended dosage can induce AKI even in systemically healthy euhydrated horses.

Phenylbutazone, nephrotoxicity, renal disease, symmetric dimethylarginine

Suxibuzone (SBZ) is a prodrug of phenylbutazone (PBZ), a nonsteroidal anti-inflammatory drug (NSAID) widely used in equine medicine. Suxibuzone is converted into active PBZ and oxyphenbutazone immediately after its absorption (Delbeke et al. 1993). It is used instead of PBZ to circumvent local irritation of gastric mucosa after oral administration (Monreal et al. 2004).

Impairment of renal function is a well-known adverse effect of NSAIDs (Whelton 1999; Beretta et al. 2005). A decrease in the glomerular filtration rate (GFR) and renal ischaemia can lead to the development of acute kidney injury (AKI) and consequently, acute kidney disease with renal papillary necrosis (Read 1983; Whelton 1999).

Acute kidney injury in horses is defined as subclinical or clinical renal injury characterized by sudden decreases in renal blood flow, GFR, and urine output (Schott and Esser 2020; Divers 2022). The human staging system for AKI, based on the increase in serum creatinine concentration from baseline, has been adapted for use in horses (Savage et al. 2019).

Several clinicopathologic tests can be used to assess kidney function and its damage in horses. The most common one is the estimation of GFR by measurement of blood urea nitrogen and creatinine concentrations (Schott and Esser 2020). Symmetric dimethylarginine (SDMA) was recently introduced for horses as a novel serum biomarker for kidney function (Siwinska et al. 2020, 2021; Schott et al. 2021; Lo et al. 2022). In humans and adult small animals, SDMA is considered advantageous for its greater sensitivity to decreased GFR and because it is less influenced by individual variability (age, sex, muscle mass) and analytical error compared to serum creatinine (Hokamp and Nabity 2016). Urinalysis can also be used to detect renal damage. Decreased urine specific gravity may suggest impaired concentrating ability of the kidneys. Increased urine gamma-glutamyl transferase to creatinine ratio (U-GGT/Cr), fractional excretion

Address for correspondence:
Zuzana Drábková
Equine Clinic
University of Veterinary Sciences Brno
Palackého třída 1946/1, 612 42 Brno, Czech Republic
E-mail: zuzkadrabkova@seznam.cz
http://actavet.vfu.cz/

of sodium (FE_{Na}), and the presence of casts in urine sediment indicate tubular damage, whereas severe proteinuria expressed as the urine protein-to-creatinine ratio (U-Prot/Cr) indicates glomerular damage (Schott and Esser 2020).

The nephrotoxicity of SBZ and PBZ has rarely been studied, and renal damage has been reported mostly in PBZ-treated horses that were concurrently dehydrated or overdosed (Snow et al. 1981; Gunson and Soma 1983; Collins and Tyler 1984; MacAllister et al. 1993; El-Ashker et al. 2012; Raidal et al. 2014). Therefore, the aims of this study were to investigate the nephrotoxicity of SBZ using the recommended dose in euhydrated horses and to compare several laboratory indicators of AKI.

Materials and Methods

This prospective study was conducted at the Equine Clinic of the University of Veterinary Sciences Brno. The study was approved by the Ethics Committee of the University of Veterinary Sciences Brno (study number: IGA VETUNI 101/2021/FVL). Client consent was obtained.

Animals

The treatment group consisted of horses treated with SBZ at the University Equine Clinic between May and October 2021. Only horses that met the following criteria were included: 1) systemically healthy horses that did not suffer from any disease which could affect the kidneys; 2) the minimum duration of SBZ therapy was five days using the recommended dosage; 3) horses had not received any other nephrotoxic medication or infusion therapy for at least six months prior to treatment.

To be considered systemically healthy and euhydrated, horses were required to have normal findings on physical examination (heart rate, mucous membranes, capillary refill time, skin tent, jugular filling) and selected laboratory values (packed cell volume, total protein, and urine specific gravity) within reference limits.

Animals in the control group were kept in similar conditions as treated horses with light exercise or paddock turnout.

Treatment

Suxibuzone was given orally in a commercial granulated form packaged in individual sachets containing 1.5 g of suxibuzone (Danilon Equidos, Laboratorios Dr Esteve SA, Barcelona, Spain). The dosage varied slightly according to the indication and the size of the animal, but never exceeded the recommended dosage. The treatment dosage ranged from 5.3–6.3 mg/kg two times a day for the first two days and was followed by a maintenance dosage of 2.6–5.7 mg/kg two times a day or 2.9–6.3 mg/kg once a day for the next three days.

Blood and urine samples

Blood samples were collected from the jugular vein on days zero and five for haematological and biochemical analysis (total protein, sodium, blood urea nitrogen, creatinine) using ethylenediaminetetraacetic acid (EDTA), heparinized tubes (FL Medical srl, Torreglia, Italy), and serum tubes with a clot activator (Meus srl, Piove di Sacco, Italy). Heparinized and EDTA tubes were submitted for haematological and biochemical analysis to the Small Animal Clinical Laboratory of the University of Veterinary Sciences Brno (Brno, Czech Republic) without undue delays. Basic haematological indicators were determined by the Sysmex XT-2000iV Hematology Analyzer (Sysmex Corporation, Kobe, Japan). Biochemical analysis was performed using the ARCHITECT c4000 Clinical Chemistry Analyzer (Abbott Laboratories, IL, USA). Serum tubes were left to clot for several hours, and following centrifugation (3000 *g*, 5 min), the serum was harvested, transferred to plain tubes, and stored frozen (−80 °C). All serum samples were shipped together after the termination of sample collection to IDEXX Laboratories for the determination of SDMA using commercially available high-throughput immunoassay (IDEXX SDMA Test; IDEXX GmbH, Leipzig, Germany).

Urine was collected on days zero and five in a nonsterile container during spontaneous urination or via aseptic catheterisation without sedation. Examination of urine sediment, reagent strip analysis (HexaPHAN, Erba Lachema s.r.o., Brno, Czech Republic) and determination of urine specific gravity (veterinary optical refractometer) were performed immediately following sample collection. Part of the urine sample was sent to the laboratory with the corresponding blood sample. Urine protein, creatinine, gamma glutamyl transferase, and sodium were determined by biochemical analysis in the Small Animal Clinical Laboratory of the University of Veterinary Sciences Brno (Brno, Czech Republic) using the ARCHITECT c4000 Clinical Chemistry Analyzer (Abbott Laboratories). The U-Prot/Cr, U-GGT/Cr and FE_{Na} were calculated.

Blood samples were taken only from horses in the control group due to financial constraints. Blood was collected on days zero and five beginning on two randomly selected days during the study period. Placebo was not given, but physical examination was performed to ensure that horses were not showing signs of clinical dehydration.

Horses were classified as suffering from AKI based on the change in serum creatinine from baseline according to the previously published staging system (Savage et al. 2019) as follows. Stage 1: increase of 150%–199% or an absolute increase ≥ 26.5 µmol/l from baseline; Stage 2: increase of 200–299% from baseline; Stage 3: increase of ≥ 300% from baseline or an absolute value > 354 µmol/l.

Statistical analysis

The normality of the distribution of the data was analysed using Shapiro-Wilk test and by visual inspection of the data. The change in creatinine was assessed using Mann-Whitney U Test. Kendall's rank correlation was used to compare the change in creatinine to the changes in SDMA, U-GGT/Cr, FE_{Na}, and U-Prot/Cr, respectively. The data were analysed using IBM SPSS Statistics for Windows, Version 28.0. Statistical significance was set at $P < 0.05$ for all analyses.

Results

Animals

Eleven horses (8 mares, 3 geldings) treated mainly for orthopaedic or ophthalmologic disorders (most cases were admitted with lameness, keratitis or uveitis) met the inclusion criteria. Their median age was 11 years (range 5–27 years) and median weight was 530 kg (range 343–575 kg). The majority were Warmbloods (8), and the remaining three animals comprised one Lipizzaner, one Haflinger, and one Arabian.

The control group consisted of 10 healthy school horses (8 mares, 2 geldings) with a median age of 14 years (range 4–21 years) and a median weight of 560 kg (range 520–680 kg). The majority were Warmbloods (4), followed by Lipizzaners (2), Haflingers (2), and Norikers (2).

Development of AKI

The values of the day zero and day five serum creatinine as well as the calculated change in creatinine are presented in Table 1. The increase in creatinine in the group of treated horses was significantly higher than in the control group ($P = 0.002$) (Fig. 1). None of the horses in the control group developed AKI, whereas three of the 11 horses in the treatment group developed stage 1 AKI.

Table 1. Serum creatinine concentration and presence of acute kidney injury according to the adapted scoring system (Savage et al. 2019) based on the change in serum creatinine in horses treated with suxibuzone and control horses.

		D0 creatinine (µmol/l)	D5 creatinine (µmol/l)	Δ creatinine (µmol/l)	n(AKI)
Treatment group (n = 11)	Median	92	102	9	3
	Min–Max	67–112	72–173	−28–84	
	IQR	76–100	76–116	5–29	
Control group (n = 10)	Median	85	79	−6	0
	Min–Max	64–100	58–90	−21–7	
	IQR	79–90	73–85	−12–2	

D0 - day zero; D5 - day five; IQR - interquartile range; n(AKI) - number of horses with acute kidney injury

Changes in serum and urinary indicators in treated horses

Changes in the measured and calculated laboratory parameters in treated horses are presented in Table 2. Serum creatinine exceeded the reference interval (RI) on day five in one of the 11 treated horses; SDMA also exceeded the RI in this horse, as well as in one other treated horse. These horses represent 2 of the 3 horses classified as having AKI on day five of treatment. In two horses, U-GGT/Cr was already above the RI in the baseline sample. In one of them (without AKI) it normalized in the post-treatment sample, while in the other (with AKI) it increased further. The FE_{Na} was above the RI in one horse (without AKI)

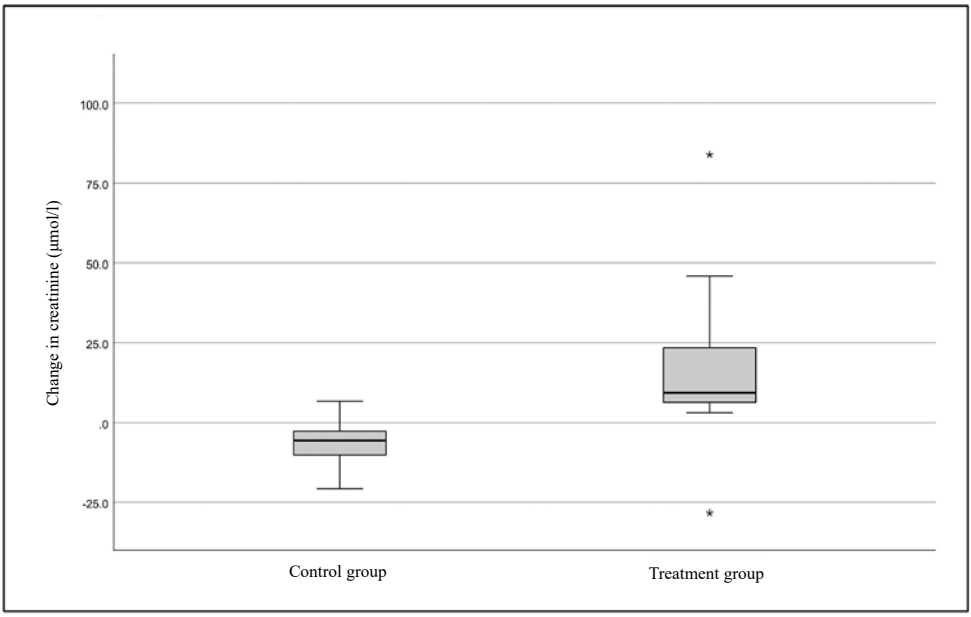

Fig. 1. Box and whisker plot representing the change in serum creatinine in the treatment and control groups. The difference between groups was significant (Mann-Whitney U Test, P = 0.002).

Table 2. Serum and urinary indicators of horses treated with suxibuzone. Samples at baseline (D0) and after 5 days of treatment (D5).

Indicator (unit) RI		D0	D0 n (AKI)	D0 n (nonAKI)	D5	D5 n (AKI)	D5 n (nonAKI)
creatinine (µmol/l) 82–156	Median	92	0	0	102	1	0
	Min–Max	67–112			72–173		
	IQR	76–100			76–117		
SDMA (µmol/l)[a] 0–0.69	Median	0.49	0	0	0.6	2	0
	Min–Max	0.45–0.69			0.45–0.94		
	IQR	0.45–0.54			0.45–0.74		
U-GGT/Cr (IU/gCr) 0–25	Median	10	1	1	9	1	0
	Min–Max	4–33			6–47		
	IQR	8–22			6–12		
FE_{Na} (%) 0–1.00	Median	0.09	0	0	0.08	0	1
	Min–Max	0.04–0.36			0.04–1.02		
	IQR	0.05–0.28			0.06–0.22		
U-Prot/Cr 0–0.50	Median	0.06	0	0	0.23	0	0
	Min–Max	0.04–0.45			0.03–0.48		
	IQR	0.05–0.16			0.05–0.31		
Changes in sediment[b]			0	0		1	2

FE_{Na} - Fractional excretion of sodium; IQR - interquartile range; n - number of samples exceeding the reference interval in horses with (AKI) or without (nonAKI) acute kidney injury; RI - reference interval; SDMA - symmetric dimethylarginine; U-GGT/Cr - gamma glutamyl transferase to creatinine ratio; U-Prot/Cr - urine protein-to-creatinine ratio

[a]SDMA results and reference interval were converted from original results (µg/dl) to SI units with conversion factor: 1 µmol/l = 20.2 µg/dl; [b]changes in sediment included haematuria and/or cast formation

after treatment, and U-Prot/Cr remained normal in all horses. Changes in urinary sediment included the presence of granular casts and erythrocytes and were found in three of the 11 treated horses, one of which met the criteria for AKI. Pigmenturia was detected by reagent strip and occurred only in horses in which the sediment was altered. Urine specific gravity remained in RI in all horses.

Correlation of laboratory parameters in treated horses

A strong correlation was found between the change in creatinine and the change in SDMA in treated horses ($\tau = 0.645$, $P = 0.012$). The changes in U-GGT/Cr ($\tau = 0.236$, $P = 0.312$), FE_{Na} ($\tau = -0.147$, $P = 0.532$), and U-Prot/Cr ($\tau = -0.073$, $P = 0.755$) were not significantly correlated with the change in creatinine.

Discussion

The results of our study suggest that both a significant increase in creatinine and changes in urinary sediment can occur in systemically healthy patients using appropriate dosing regimens even within the first five days of treatment with SBZ. This is contrary to the published opinion that renal adverse effects are not associated with the prescribed dosing regimens for PBZ unless haemodynamic risk factors exist (Raidal et al. 2014; Cook and Blikslager 2015; Divers 2022). Because SBZ is converted to PBZ immediately after absorption, it is likely that the results of our study can be extrapolated to treatment with PBZ.

The use of the change in creatinine rather than a single creatinine value to assess kidney status is supported by our results. A significant decrease in GFR would have gone undetected in two of the three horses with AKI in this study if only a single creatinine value had been assessed. This is in agreement with previously published recommendations (Hokamp and Nabity 2016; Schott and Esser 2020; Galen et al. 2022).

The change in SDMA correlated with the change in creatinine in our study. Notably, in one horse with AKI, SDMA exceeded the RI while creatinine remained within the RI. This corresponds with the reported finding that SDMA detects decreased kidney function earlier than creatinine (Lo et al. 2020; Schott and Esser 2020). Therefore, if a basal creatinine sample was not collected, SDMA might be more reliable than creatinine as a single monitoring parameter for AKI during treatment with SBZ or PBZ.

No significant correlation was found between the change in creatinine and the other monitored indicators. U-GGT/Cr is an indicator of damage to tubular cells and was considered more sensitive than serum creatinine in gentamicin-induced renal damage (Hinchcliff et al. 1989). NSAIDs do not induce tubular damage directly, but rather as a delayed consequence of reduced renal perfusion (Whelton 1999). This delay in the onset of tubular damage may be the reason why U-GGT/Cr did not correlate with the change in creatinine in our study, and consequently it is not an appropriate monitoring parameter for early NSAID-induced renal damage. However, in a study of PBZ-induced nephrotoxicity in which the renal damage in the studied population was more severe, U-GGT/Cr and U-Prot/Cr proved to be useful monitoring parameters for AKI (El-Ashker et al 2012).

The main limitation of this study was the small sample size due to the low number of horses that met the inclusion criteria during the study period. Nevertheless, our results indicate that treatment with SBZ and PBZ is not without consequences. Although the study period was too short to judge the long-term effects of these drugs in treated horses, we strongly recommend monitoring the renal function and hydration status during treatment with SBZ or PBZ. More robust studies are warranted to confirm these conclusions.

Acknowledgements

This work was supported by the Internal Grant Agency of the University of Veterinary Sciences Brno (study number: IGA VETUNI 101/2021/FVL).

References

Beretta C, Garavaglia G, Cavalli M 2005: COX-1 and COX-2 inhibition in horse blood phenylbutazone, flunixin, carprofen and meloxicam: an *in vitro* analysis. Pharmacol Res **52**: 302-306

Collins LG, Tyler DE 1984: Phenylbutazone toxicosis in the horse: a clinical study. J Am Vet Med A **184**: 699-703

Cook VL, Blikslager AT 2015: The use of nonsteroidal anti-inflammatory drugs in critically ill horses. J Vet Emerg Crit Care **25**: 76-88

Delbeke FT, Vynckier L, Debackere M 1993: The disposition of suxibuzone in the horse. J Vet Pharmacol Ther **16**: 283-290

Divers TJ 2022: Acute kidney injury and renal failure in horses. Vet Clin N Am-Equine **38**: 13-24

El-Ashker MR, Hussein HS, El-Sebaei MG 2012: Evaluation of urinary variables as diagnostic indicators of acute kidney injury in Egyptian draft horses treated with phenylbutazone therapy. J Equine Vet Sci **32**: 268-273

Galen G, Olsen E, Siwinska N 2022: Biomarkers of kidney disease in horses: a review of the current literature. Animals **12**: 2678

Gunson DE, Soma LR 1983: Renal papillary necrosis in horses after phenylbutazone and water deprivation. Vet Pathol **20**: 603-610

Hinchcliff KW, McGuirk SM, MacWilliams PS, Cooley AJ 1989: Phenolsulfonphthalein pharmacokinetics and renal morphologic changes in adult pony mares with gentamicin-induced nephrotoxicosis. Am J Vet Res **50**: 1848-1853

Hokamp JA, Nabity MB 2016: Renal biomarkers in domestic species. Vet Clin Path **45**: 28-56

Lo HC, Winter JC, Merle R, Gehlen H 2022: Symmetric dimethylarginine and renal function analysis in horses with dehydration. Equine Vet J **54**: 670-678

MacAllister CG, Morgan SJ, Borne AT, Pollet RA 1993: Comparison of adverse effects of phenylbutazone, flunixin meglumine, and ketoprofen in horses. J Am Vet Med A **202**: 71-77

Monreal L, Sabaté D, Segura D, Mayós I, Homedes J 2004: Lower gastric ulcerogenic effect of suxibuzone compared to phenylbutazone when administered orally to horses. Res Vet Sci **76**: 145-149

Raidal SL, Hughes KJ, Charman AL, Nielsen SG, Phillips, JK, Noble GK 2014: Effects of meloxicam and phenylbutazone on renal responses to furosemide, dobutamine, and exercise in horses. Am J Vet Res **75**: 668-679

Read WK 1983: Renal medullary crest necrosis associated with phenylbutazone therapy in horses. Vet Pathol **20**: 662-669

Savage VL, Marr CM, Bailey M, Smith S 2019: Prevalence of acute kidney injury in a population of hospitalized horses. J Vet Intern Med **33**: 2294-2301

Schott HC, Esser MM 2020: The sick adult horse: renal clinical pathologic testing and urinalysis. Vet Clin N Am-Equine **36**: 121-134

Schott HC, Gallant LR, Coyne M, Murphy R, Cross J, Strong-Townsend M, Szlosek D, Yerramilli M, Li J 2021: Symmetric dimethylarginine and creatinine concentrations in serum of healthy draft horses. J Vet Intern Med **35**: 1147-1154

Siwinska N, Zak A, Slowikowska M, Niedzwiedz A, Paslawska U 2020: Serum symmetric dimethylarginine concentration in healthy horses and horses with acute kidney injury. BMC Vet Res **16**: 1-6

Siwinska N, Zak A, Paslawska U 2021: Detecting acute kidney injury in horses by measuring the concentration of symmetric dimethylarginine in serum. Acta Vet Scand **63**: 1-10

Snow DH, Douglas TA, Thompson H, Parkins JJ, Holmes PH 1981: Phenylbutazone toxicosis in equidae: a biochemical and pathophysiological study. Am J Vet Res **42**: 1754-1759

Whelton A 1999: Nephrotoxicity of nonsteroidal anti-inflammatory drugs: physiologic foundations and clinical implications. Am J Med **106**: 13S-24S