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Original Research

Subspecies Studies: Pharmacokinetics and Pharmacodynamics of a Single Intravenous Dose of Xylazine in Adult Mules and Adult Haflinger Horses

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ABSTRACT

This study reveals the different effectiveness of xylazine in mules compared with horses. Fourteen adult mules (mean body weight ± standard deviation, 466 ± 89 kg) and six adult Haflinger horses (483 ± 39 kg) chosen from a single livestock operation in Germany received 0.6 mg of the α_2-agonist xylazine administered intravenously per kilogram of body weight. Principal pharmacokinetic and pharmacodynamic parameters were determined while the animals received a routine dental treatment. To objectively assess the depth of sedation, a variety of behavioral and clinical parameters were assessed and transferred to a scaled score system. Compared with the Haflinger horses, the depth of sedation in mules differed significantly between 10 and 45 minutes after xylazine administration. In the mule, sedation was good during the first 10 minutes, moderate at 15 minutes, and insufficient at 30 minutes. In the horse, sedation was excellent during the first 15 minutes, moderate at 30 minutes, and insufficient at 45 minutes. Moreover, significant ($P < .05$) subspecies differences in the pharmacokinetics of xylazine were detected between the mules and the horses. Data analysis followed the two-compartment model, which had a correlation with the measured data of $R^2 = .99$. Values for $t_{1/2\beta}$ (half-life during elimination), mean residence time, mean residence time$_{(0\text{-}tz)}$ (residence time on last measuring time point above limit of quantification), k_{21} (velocity constant for distribution from peripheral to central compartment), β (velocity constant during elimination), and B (relative y-intercept) varied significantly between the two subspecies.

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1. Introduction

The mule (female horse × male donkey) unifies special qualities of the donkey with the strength and power of the horse. Passed down by the desert-accustomed donkey, the mule possesses the ability to better cope with extreme temperatures. Also, it recovers from exposure to extreme heat much faster than the horse. Mules live long, are less demanding in nutrition than the horse, are sober minded in case of danger, and are generally more sure footed in rougher terrain than the horse. Humans have bred mules for >3,000 years [1,2], and the worldwide population of approximately 10.8 million mules [3] emphasizes the importance of this hybrid species for man until today.

The physiology of the *Equidae* species donkey, horse, and mule generates differences in drug distribution, metabolism, and elimination, and may result in either toxicity or lack of efficiency. Significant differences in the pharmacokinetics (PK) and pharmacodynamics (PD) of several intravenously administered drugs have been reported from comparison studies between horses, donkeys, and mules [4-11]. Investigations of anesthetic drug combinations in donkeys and mules [5,6,10,11] have demonstrated the necessity to find adequate anesthetic

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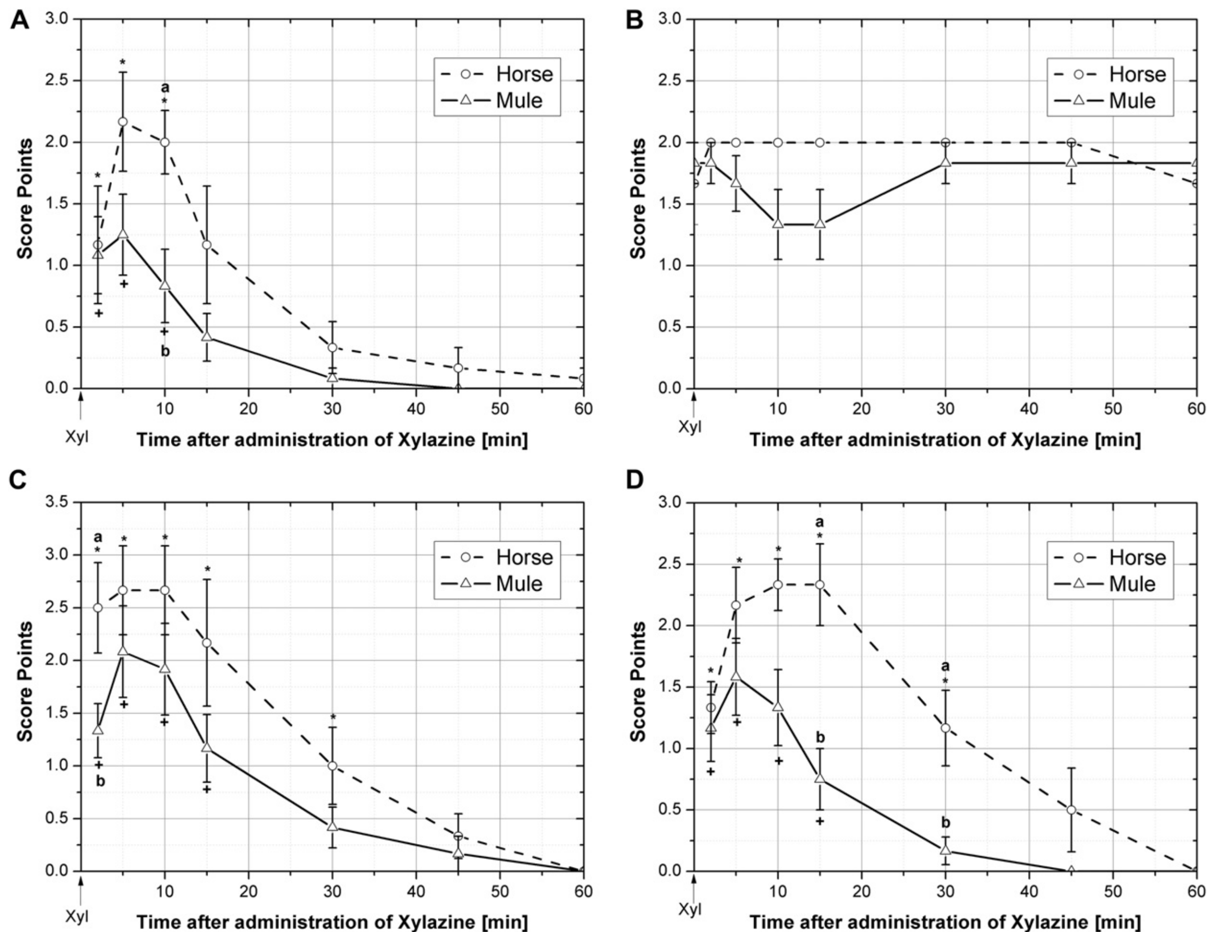

Fig. 1. (**A**) standing ability; (**B**) behavior during dental treatment; (**C**) head height; (**D**) ear tone; (**E**) drooping of the lower lip; (**F**) sensibility on the ear fringe; (**G**) reaction to auditory stimulus; (**H**) reaction to visual stimulus. a differs significantly from b ($P < .05$); *differs significantly from mean initial group value ($P < .05$; horse); + differs significantly from mean initial group value with ($P < .05$; mule).

regimens for each of this species. Differences between species in PK and PD of a given drug are numerous and frequently unpredictable [4]. Although a mule is genetically half a donkey, its physiological response to drug administration is not predictably halfway between a donkey and a horse. However, documentation of the responses of mules to various injectable anesthetics is very limited, and pharmacokinetics of the α_2-agonist xylazine in mules apparently have never been researched before this study.

This study was designed to yield combined PK and PD of xylazine in mules under field conditions, as an isolated examination of the pharmacokinetic data of a drug is not regarded to be reasonable [12]. All animals were sedated to facilitate the floating of teeth, which is a procedure that usually provokes pronounced behavioral responses up to defending movements when the level of sedation is insufficient. Considering the dynamics of the clinical procedure, pharmacodynamic and pharmacokinetic parameters of this study had to be evaluated.

2. Materials and Methods

The study used six Haflinger horses and 14 mules (two female mules in a prestudy assessment, six female mules [Mf], and six male mules [Mm]) aged 5-26 years. The study itself was performed in early summer of 2007 in Bad Reichenhall, Germany. No animals had been added to this stock later than 2 years before this study. The animals were kept under the same keeping, feeding, and working conditions. All of them were adult, healthy, working animals in good condition, with a physiological body weight. They were regularly dewormed and vaccinated (equine influenza, equine herpes virus (EHV-1 + EHV-4), tetanus, rabies). Blood parameters, especially those of the liver and the kidney, had been determined to ensure organic normality. The animals needed to be sedated for routine tooth rasping. Xylazine hydrochloride (Rompun, Bayer Vital GmbH, Leverkusen, Germany) was aspirated into a sterile disposable 20-mL syringe, and 0.6 mg of xylazine per kilogram of body weight was administered slowly during 10 seconds into the left jugular vein by a 4/G12-gauge catheter (Braunüle MT Luer Lock sterile, 8 cm, Braun Melsungen AG, Melsungen, Germany) stitched to the skin. After administration, blood was aspirated into the syringe three times to avoid potential residues of xylazine in the catheter or syringe. After disposal of the syringe, the catheter was locked with a corresponding sterile Luer-Lock. Approximately 5 minutes after administration, a mouth speculum

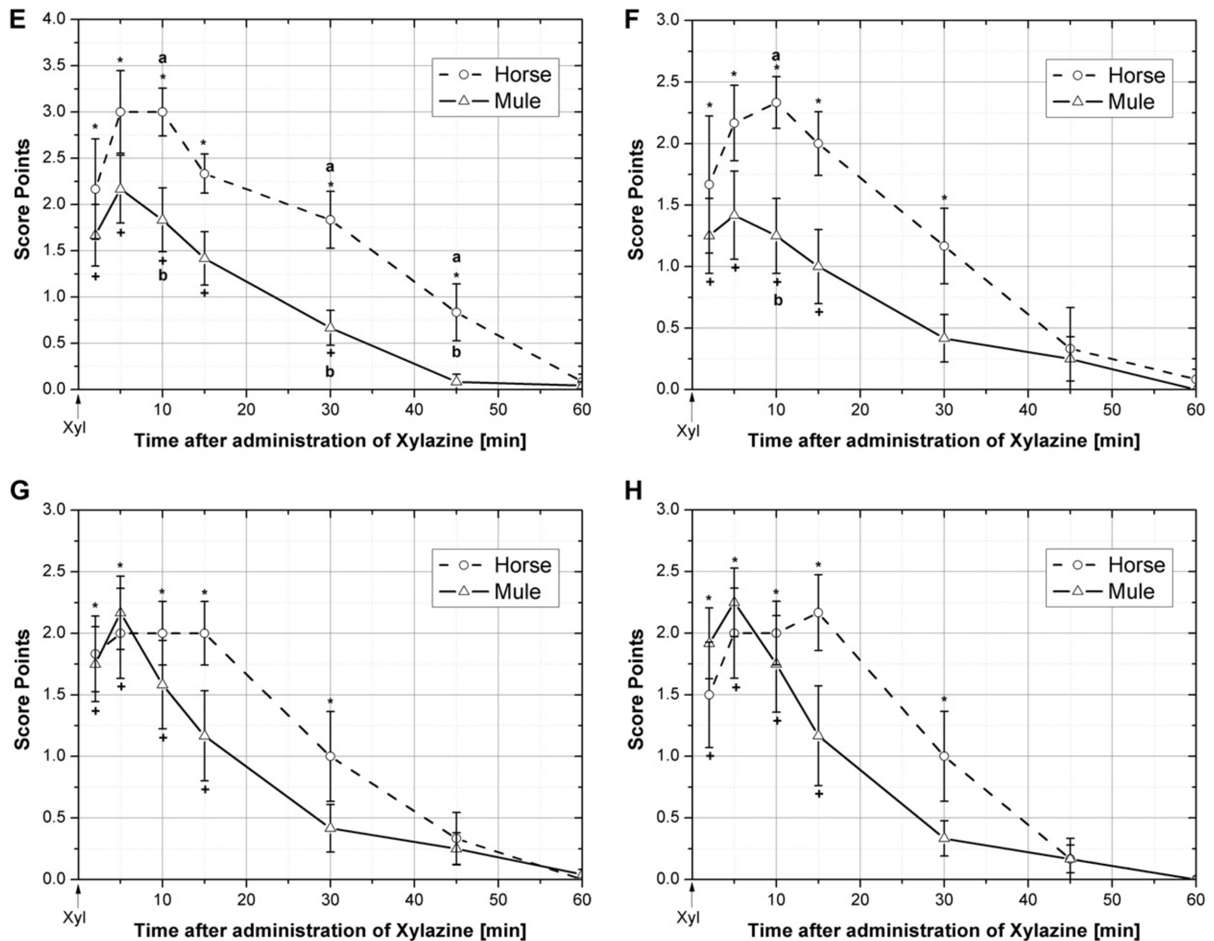

Fig. 1. (*continued*).

was inserted. The procedure of floating the teeth was performed with both powered and manual equipment. All animals selected needed minor to medium floating of their teeth. In all cases, the procedure was stopped at 10 minutes for scoring and collecting of blood samples and taken to an end before 15 minutes after xylazine administration.

2.1. Pharmacodynamics of Xylazine by Means of a Clinical Score Point System

Clinical measurements designed to reflect the degree of sedation were recorded before sedation (0) as well as 2, 5, 10, 15, 30, 45, 60, 75, 90, 105, 120, 180, 240, and 480 minutes after xylazine administration. A further observation 24 hours after xylazine administration was made in some cases and always confirmed the baseline values. To minimize side effects, parameters were always measured by the same person exactly in the following order: (1) standing ability, (2) behavior, (3) head height, (4) ear tone and pricking up of the ears, (5) drooping and trembling of the lower lip, (7) collecting of blood samples, (8) heart rate, (9) sensibility on the ear fringes, (10) rectal body temperature, (11) respiratory rate, (12) auditory stimulation, (13) visual stimulation, (14) transpiration, (15) nociceptive stimulus, and (16) penile prolapse.

The pharmacodynamic effects of xylazine were assessed with the help of a score point (SP) system. Only those clinical parameters that proved to be representative for the measurable clinical impacts were used. These were standing ability, behavior, head height, ear tone, drooping of the lower lip, sensibility on the ear fringes, and reaction to auditory and visual stimulus. Mean group SPs of each clinical parameter were added at each measuring time point to determine the degree of sedation in SPs as follows: 0-5 = insufficient sedation, >5-10 = moderate sedation, >10-15 = good sedation, and >15-20 = excellent sedation.

2.1.1. Behavioral Parameters Used for the SP System

Standing ability (Fig. 1A): this was scored on a scale from 0 to 3 and naturally depicted the degree of ataxia. A score of 0 was given if the animal was not or did not seem to be sedated. An animal swaying slightly but still resting stable received a score of 1. A score of 2 represented an animal swaying considerably. Often, the hind legs were crossed and the forelegs buckled. A score of 3 was assigned to an animal in manifest disequilibrium (Table 1).

Behavior (Fig. 1B): this was recorded in two degrees. Animals that rested easily received a score of 2. Agitation and nervous behavior was scored with zero SPs (Table 1).

Table 1
Score points for behavioral parameter scoring

Points	Standing Ability	Behavior	Head Height
0	No sedation	Nervous	Shoulder joint
1	Swaying lightly		Between shoulder and elbow
2	Swaying considerably	Resting easily	Below elbow, but clearly above carpus
3	Disequilibrium		Near carpus
4			Clearly below carpus

Points	Ear Tone	Lower Lip
0	Normal interest	Lips closed
1	Reduced interest	Lips closed, trembling
2	Markedly reduced interest, ears slightly drooping	Lips slightly opened, trembling
3	Apathy/drooping of the ears/tone reduced	Lips >1 cm apart, trembling
4		Atonic, trembling

Points	Ear Fringes	Auditory	Visual
0	Equal/more initial value	Individual reaction	Individual reaction
1	Reduced reaction	Subdued reaction	Subdued reaction
2	Strongly reduced reaction	Strongly subdued reaction	Strongly subdued reaction
3	No reaction	No reaction	No reaction
4			

Head height (Fig. 1C): shortly after the administration of xylazine, equids typically show a lowering of the head [13-15] that gradually normalizes during the fading of the sedation. The head height of each animal was evaluated from a lateral position. A virtual horizontal line was drawn from under the muzzle to characteristic bone marks. Scoring was as follows: 0 points = muzzle at the height of the shoulder joint (art. glenohumeralis), 1 point = height of the muzzle between the shoulder and the elbow joint (lower/equal art. glenohumeralis and higher/equal art. cubiti); 2 points = height of the muzzle below the elbow (art. cubiti) but clearly above the carpus (art. carpi); 3 points = height of the muzzle in the area of art. carpi; 4 points = muzzle clearly below art. carpi (Table 1).

Ear tone and pricking up of the ears (Fig. 1D): horses and other equids usually show their interest in surroundings by movements of the eyes and ears as well as by specific movements of the head. In this study, movement of the eyes and ears were provoked by defined visual and acoustic stimuli. Preanesthetic tone and movement of the ears without defined stimulus was categorized as follows: 0 points = normal interest in surroundings; 1 point = reduced interest in surroundings, but manifest pricking up of the ears; 2 points = markedly reduced interest in surroundings and slight drooping of the ears; 3 points = strongly reduced interest in surroundings/apathy, drooping of the ears, and reduced ear tone (Table 1).

Drooping and trembling of the lower lip (Fig. 1E): These also become manifest in equines when sedated with α_2 agonists. SPs were assigned as follows: 0 points = lips closed, no trembling; 1 point = lips closed but trembling; 2 points = lower lip slightly opened, possibly trembling; 3 points = lower lip clearly opened (gap to upper lip, at least 1 cm), possibly trembling; 4 points = lower lip atonic, possibly trembling (Table 1).

Ear fringes (Fig. 1F): a light stroke on the ear fringe served as tactile stimulus. Stroking was done from a position next to the front leg so as not to disturb the effect by stimulating the animal visually. All animals showed a reaction and were classified based on their individual baseline reaction as follows: 0 points = preanesthetic value/increased reactions (turning away of the head or the ear, shaking of the head); 1 point = reduced reaction (slightly slower or less intensive reaction); 2 points = strongly reduced reaction (only slight movement of the ear); 3 points = no reaction (Table 1).

Auditory stimulation (Fig. 1G): an auditory stimulus was set with a tin can filled with horse treats. From a position approximately 1 m in front of the animals' head, the tin can was shaken behind the back of the person examining. For each animal, the individual reaction was set as baseline value before xylazine administration. Attenuation of this reaction was transferred into SPs as follows: 0 points = individual reaction (ie, rapid movement of eyes/ears/head in direction of the noise, snuffling); 1 point = subdued reaction (ie, only ear/eye movement, slow movement of the head); 2 points = strongly subdued reaction (ie, slow movement of an ear); 3 points = no reaction at all (Table 1).

Visual stimulation (Fig. 1H): a visual stimulus was set with an unfolded canvas bag presented with both hands from a position approximately 1 m in front of the animal's head. For each animal, the individual reaction was set as baseline value before administration of xylazine. Attenuation of this reaction was transferred into SPs as follows: 0 points = individual reaction (ie, rapid movement of eyes/ears/head in direction of the bag, snuffling, or a startled reaction under sedation); 1 point = subdued reaction (ie, only ear/eye movement, slow movement of the head); 2 points = strongly subdued reaction (ie, slow movement of an ear); 3 points = no reaction at all (Table 1).

2.1.2. *Physiological Parameters*

Heart rate (Fig. 2A): baseline recordings of heart rate were made by auscultation of the heart for 1 minute. Position of auscultation was the fifth intercostal gap on the left side of the thorax, approximately 6 inches behind the olecranon.

Rectal body temperature (Fig. 2B): this was measured with a digital thermometer (DiGItemp TM, Servoprax R GmbH, Wesel, Germany) during the whole study.

Respiratory rate (Fig. 2C): recordings of respiratory rate (Fig. 2C) were made by visually counting respiration for 30 seconds from a laterocaudal position while measuring the rectal temperature.

2.1.3. *Additional Observations and Findings*

Transpiration (Fig. 3A): the location and intensity of transpiration after the administration of xylazine was recorded as follows: 0 points = no transpiration; 1 point = very slight transpiration (ie, on the ear base or between the front legs); 2 points = slight transpiration on different parts of the body (ie, on the forehead, on the ear bases, between the front legs, and between the hind legs); 3 points = some dark sweating spots or dripping off of little sweat beads; 4 points = multiple dark sweating spots (ie, forehead, ear base, crest line under the mane, flank, and loin).

Nociceptive stimulus: because mules are extremely intelligent and kick with excellent aim without warning [16], we refrained from using an electric impulse generator. Instead, a defined pinch in the Mm pectorales superficiales served as an attenuated nociceptive stimulus.

Fig. 2. (**A**) heart rate; (**B**) body temperature; (**C**) respiratory effects of xylazine. *differs significantly from mean initial group value ($P < .05$; horse); + differs significantly from mean initial group value ($P < .05$; mule).

Penile prolapse (Fig. 3B): for male animals, the degree of penile prolapse was recorded as follows: 0 = no prolapse, 1 = slight prolapse of a few centimeters, 2 = prolapse of 10-15 cm, 3 = manifest prolapse of at least 15 cm.

Urination was noted in minutes after the administration of xylazine.

Outstanding peculiarities: other observations not fitting in these categories were noted in the protocol. For example, some animals showed a manifest drooping of the eyelids.

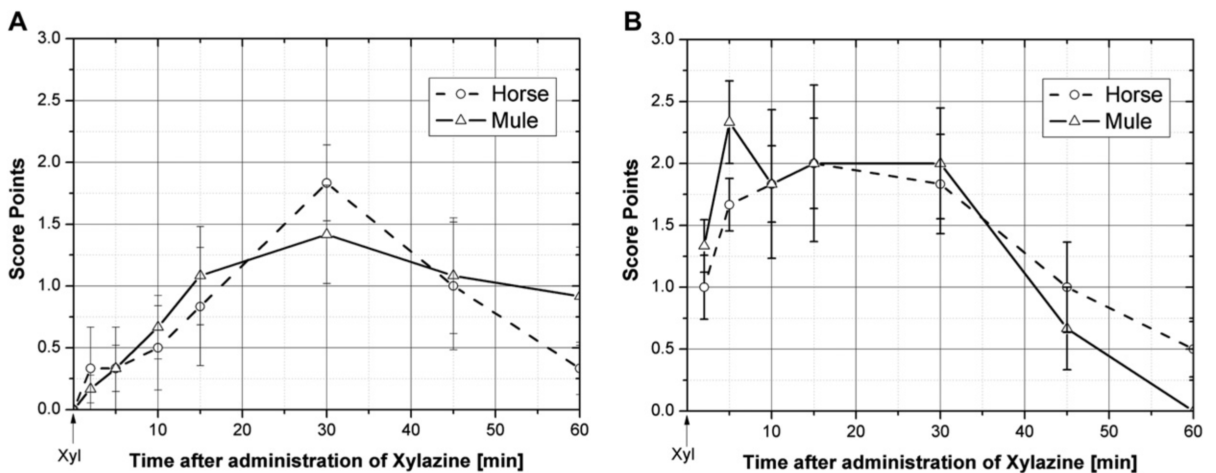

Fig. 3. (**A**) transpiration; (**B**) penile prolapse.

2.2. Pharmacokinetics of Xylazine by Means of Quantitative Determination in Plasma

2.2.1. Collection of Blood Samples

Blood samples (10 mL) were drawn from the indwelling jugular catheter into ethylenediaminetetraacetic acid tubes (BD Vacutainer, K2E 18 mg, PLUS, 10 mL, 16 × 100 mm) for determining xylazine concentration before drug administration and at 2, 5, 10, 15, 30, 45, 60, 75, 90, 105, and 120 minutes (when scoring the PD parameters) as well as 3, 4, 8, and 24 hours after drug administration. The tubes were inverted five times and centrifuged for 5 minutes at 3000 rpm (Rotanta/S, Hettich Zentrifugen, Tuttlingen, Germany, fourfold swing-out rotor, 4 × 90 g) within 60 minutes of sample collection. The plasma fraction was transferred into transportation tubes (3.5 mL, 55 × 12 mm, Sarstedt AG und Co., Nüembrecht, Germany) and frozen at −20°C until assay.

2.2.2. Assay for Xylazine Analysis

Plasma concentrations of xylazine were measured by an adapted original high-performance liquid chromatographic method [17] using the internal standard method of calibration by peak area. The validated analytical method reached a limit of determination of 1.5 ng/mL and a limit of quantification of 5 ng/mL of plasma. Recovery rates for xylazine (Sigma Aldrich Chemie GmbH, Taufkirchen, Germany) from spiked plasma standards were 90.99 % ± 5.90 % (mean ± standard deviation). The main steps of the procedure were as follows: after addition of internal standard (500 ng of clonidine, Sigma Aldrich Chemie GmbH) to the aliquots of plasma (1 mL), the sample was vortexed (Tubes Shaker, Renner, Darmstadt, Germany) for 5 seconds underneath a flue, and 1 mL of 2 N NaOH was added. Then, the capped sample was shaken with ether (5 mL) for 60 seconds. Centrifugation for 5 minutes at 6°C and 2 rpm (Minifuge RF, Heraeus, Hanau, Germany) was followed by a freezing time of 30 minutes at −20°C to separate the organic phase from the plasma fraction. The organic phase was transfused into a new tube and then evaporated (15 minutes, medium drying rate, Speed Vac Plus SC210A Savant, Freezemobile 12SL Virtis Sentry Savant Instruments Inc., Farmingdale, NY). After redissolving the sample in 200 μL of a phosphate buffer (Meyer buffer [18,19]), 100 μL of the sample was injected into the high-performance liquid chromatography system (Beckman Systems Gold, 19 MPa, LiChroSorb RP-select B [5 μL] Hibar RT 250-4, Merck, Darmstadt, Germany). The ultraviolet detector was operated at a wavelength of 225 nm.

2.3. Mathematical and Statistical Methods

Pharmacokinetic data was calculated for each animal using PK Analyst (version 1.0, MicroMath Scientific Software, Salt Lake City, UT), based on the two-compartment open model. This is a long established standard model for xylazine also used by other authors [20,21] for the calculation of pharmacokinetics of xylazine in the plasma of horses and ponies using the biexponential equation (1).

$$C^t = A \times e^{-\alpha \times t} + B^{-\beta \times t} \quad (1)$$

Here, C^t stands for the plasma xylazine concentration (ng/mL) at time t (minutes), A and B are the extrapolated zero-time intercepts of the distribution/elimination curves, and α and β represent velocity constant during distribution/elimination.

Calculated data, measurements of plasma concentration, and clinical data were statistically analyzed using SPSS for Windows (SPSS Inc., Chicago, IL) and GraphPad Prism (Prism 5 for Windows, GraphPad Software Inc., La Jolla, CA). Level of significance in all tests was defined with an error probability of $P < .05$.

First, data were tested for normality using the Kolmogorov–Smirnov normality test under correction of significance by the Dallal–Wilkinson–Lilliefor method for *P*. Second, data were analyzed for homogeneity of variance using Levene test. In case of homoscedasticity, normally distributed data were tested with a two-tailed *t* test. Subspecies differences for normally distributed data were tested with an unpaired *t* test. Groups were tested for significance to their baseline values with a paired *t* test in case of normally distributed data and with Wilcoxon rank sum test in case of non-normally distributed data (significant differences to the baseline value [$P < .05$] marked with * for horses and + for mules in Fig. 1 and Fig. 2). Subspecies differences of non-normally distributed data were tested using Whitney–Mann rank sum test. Significant differences between mules and horses ($P < .05$) are marked with a/b (all horses/all mules), a/c (all horses/female mules), and a/d (all horses/male mules). No significant differences were to be found between the group of male and female mules.

3. Results

3.1. Mean Body Weight of the Animals Used in This Study

The original three groups had a similar mean body weight: Haflinger horse (H) group, 483 ± 39 kg (mean ± standard deviation, n = 6), female mules in Mf group, 475 ± 104 kg (n = 8), and male mules in Mm group, 453 ± 73 kg (n = 6). However, dividing the mules in two groups according to gender turned out to be unnecessary. To obtain statistically more adequate results for this rare subspecies, male and female mules were merged into one group (M). Because for 2 of the female mules, pharmacodynamic data was insufficient, the M group was formed by 12 mules for the pharmacodynamic assessment and 14 mules for the pharmacokinetic calculations.

3.2. Pharmacodynamics of Xylazine

3.2.1. Behavioral Parameters Used for the SP System

Standing ability: all animals showed a disturbance in their standing ability. However, disturbances of equilibrium in the H group reached higher scores and were of longer duration than in the M group. Both groups showed significant disturbances compared with their baseline values during the first 15 minutes after the administration of xylazine. A statistically significant difference in parameter value was obtained 10 minutes after xylazine administration (Fig. 1A: value a differs significantly from value b, $P < .05$).

Behavior (Fig. 1B): after a short period of adaptation, all animals stood calm and relaxed next to the person holding them by the rein before the beginning of the study. In the H group, all patients stayed calm during the dental treatment. In the M group, some patients showed defensive movements with the head, efforts to break away, and scurrying with the legs starting with the beginning of the dental treatment with a ceiling effect between 10 and 15 minutes after xylazine administration.

Head height (Fig. 1C): lowering of the head was faster in the H group. Although all animals showed a significant lower head height during 2-15 minutes after the administration of xylazine, in the H group, a significant lower head height persisted until 30 minutes after administration. Head height in the M was higher, with a significant difference from the H group 2 minutes after the administration of xylazine.

Ear tone and pricking up of the ears (Fig. 1D): animals in the H group showed an obvious reduction in ear tone at 5 minutes, with the maximal degree of atony at 15 minutes. Significant differences between the M and H groups were between 15 and 30 minutes after administration. Mules showed a maximal reduction in ear tone at 5 minutes and never reached the degree of ear atony of the H group.

Drooping of the lower lip (Fig. 1E): under xylazine administration, all animals showed a quick reduction in the tone of the lower lip. Parameter values were significant compared with baseline values in the H group from 2 to 45 minutes and in the M group from 2 to 30 minutes after the administration of xylazine. In the M group, drooping of the lower lip and loss of tone were less pronounced, with significant difference from the H group at 10, 30, and 45 minutes after administration.

Ear fringes (Fig. 1F): reaction to stroking the ear fringe was significantly subdued in all animals. In the H group, SPs were significantly lower than the initial values from 2 to 30 minutes after the administration of xylazine. In the M group, reaction was significantly subdued from 2 to 15 minutes, although the reduction of the individual reaction was less intense than in the H group.

Reaction curves were similar for both groups, but shifted by about 15 minutes, as the M group recovered to baseline values approximately 15 minutes earlier than the H group. At 10 minutes after xylazine administration, reaction of the M group was significantly ($P < .05$) more intense than the H group.

Reaction to auditory stimulation (Fig. 1G): in both groups, reaction to an auditory stimulus was clearly subdued shortly after the administration of xylazine. In the M group, the onset of response to auditory stimulation was noticed at 10 minutes after xylazine administration, whereas in the H group, an equal reaction was observed at 15 minutes after administration. Compared with the reaction at the point of departure (zero), the reaction in the M group was significantly ($P < .05$) subdued between 2 and 15 minutes after administration, whereas in the H group, the reaction was significantly subdued between 3 and 30 minutes after xylazine administration.

Reaction to visual stimulation (Fig. 1H): shortly after the administration of xylazine, reaction to the visual stimulus in all animals was clearly subdued or not triggered at all. In the M, the onset of the reaction to visual stimulation was at 10 minutes after the administration of xylazine. Reaction was significantly ($P < .05$) subdued from 2 to 15 minutes. In the H group, the cushioning effect of xylazine prevailed until 30 minutes after the administration of xylazine. Reaction was significantly subdued during the first half of an hour (2-30 minutes).

3.2.2. Physiological Parameters

Heart rate (Fig. 2A): in both groups, heart rate at the measuring time points was compared with the individual baseline values. In the M group, a significant lowering of heart rate was observed from 2 to 10 minutes after the administration of xylazine. The deepest lowering of heart rate was observed at 2 minutes after administration, with a mean of 83% of individual baseline values. The quick increase in heart rate starting at 5-10 minutes (85% and 90% of the baseline values), and accessing the individual baseline values at 15 minutes, seems to be due to stimulation by the floating procedure. Without stimulation, heart rate in the M group was 93% of the baseline values at 30 minutes and almost equal to baseline values at $\geq$45 minutes.

In the H group, significant lowering of heart rate was perceived during the first 2 hours. The deepest values were measured at 2 and 5 minutes after administration (65% of baseline values). At 10 minutes, values were at 74% of the baseline values and increased to 75% at 15 minutes.

Body temperature (Fig. 2B): measurement of rectal body temperature was at no time point significantly different from the baseline values. Tendency was an initial slight increase, followed by a decrease in body temperature until 45 minutes in the H group and 90 minutes in the M group.

Respiratory rate (Fig. 2C): mean baseline values for respiratory rate in both the M and the H group were 16 per minute. For statistical calculations, respiratory rate was stated in percentage of the initial individual values. In the M group, a significant decrease in respiratory rate was measured at 2 minutes (91%). At this same time point, in the H group, respiratory rate was at 66% of the initial values and remained significantly lower for the following 3 hours. At 180 minutes, the respiratory rate in the H group reached not more than 77% of the initial values. Baseline respiratory rate in the H group was not reached until >8 hours after the administration of xylazine. During the whole first hour, there was a significant ($P < .05$) difference in the decrease of respiratory rate between the M and the H group.

3.2.3. Additional Observations and Findings

Transpiration (Fig. 3A): no species differences in the degree of transpiration were observed. The maximal degree of sweating was at 30 minutes after the administration of xylazine. In the mean, each animal showed a moderate sudation on different parts of the body (forehead, ear base, between the front/hind legs).

Nociceptive stimulus: setting a nociceptive stimulus was only successful in the H group, with an evident reduction of the reaction between 2 and 5 minutes after the administration of xylazine. In the M group, there was a big variation in the responses to this stimulus: some mules ignored the pinch, some showed a clear adaptation, and some increased their defensive reaction to this stimulus; therefore, the stimulus could not be used for the clinical score.

Degree of penile prolapse (Fig. 3B): a manifest penile prolapse of at least several centimetres was observed during 2-45 minutes after the administration of xylazine in all male mules, with a maximum between 15 and 30 minutes. No species differences between male mules and male horses were observed.

Urination: during the first hour after the administration of xylazine, two animals of the H group (A and B each 1×) and 4 of the M group (mule I and II, each 1×; mule III and VI each 2×) urinated.

Outstanding peculiarities: at the time of the general examination preceding the study, none of the animals showed irregularities in the respiratory tract or the heart. However, under the influence of xylazine, some of the animals showed temporarily reversible changes, such as heart murmurs or respiration synchronous stridors.

One of the horses showed a pronounced inspiratory and expiratory dyspnea with a loud snoring breath from 10 to 15 minutes after the administration of xylazine. The latter was diagnosable in an alleviated form until 45 minutes after the administration of xylazine.

Another horse showed a clear arrhythmia of the heart between 2 and 5 minutes after the administration of xylazine. The case was the same in one of the male mules, which additionally showed a systolic heart murmur of second to third degree at 5 minutes and first degree at 10 minutes, which was no longer diagnosable after another 5 minutes.

Similarly, another male mule showed arrhythmic heart beats and a pronounced hanging of the eyelids at 2 minutes. At 5 minutes, the heart beat in this animal was rhythmic and regular again.

A female mule showed a systolic heart murmur of second degree at 2 and 5 minutes.

Another male mule showed a clearly irregular heart beat at 2 minutes, which prevailed in an alleviated form until 15 minutes and then vanished.

In Figure 4, mean group values of the H and M group are graphed in an x-y diagram. Mean group values of the degree of sedation of the M group were significantly ($P < .05$) different at 10, 15, 30, and 45 minutes after the administration of xylazine. The resulting curves with summarized SPs of behavior, standing ability, head height, drooping of the ear and of the lower lip, sensibility of the ear, and reaction to auditory and visual stimulus depict the clinical manifestation of xylazine. Value a differs significantly from value b ($P < .05$). The degree of sedation was classified as follows: 0-5 SP = insufficient sedation, >5-10 = moderate sedation, >10-15 = good sedation, >15-20 = excellent sedation.

3.3. Results of Assay for Analysis of Xylazine in Plasma

The concentration for xylazine in Meyer buffer was linear throughout the range from 2000 ng/100 to 1.95 ng/100 μL. For the concentration of clonidine in Meyer buffer, calibration curve was linear from 1500 ng/100 to 187.5 ng/100 μL. Correlation for both calibration curves showed an R^2 value of .99 after linear regression analysis. For linearity of calibration curves (xylazine and clonidine in plasma), the ratios of peak areas of xylazine in plasma versus internal standard clonidine in plasma were graphed in relation to concentration and, on all days of assay, reached a coefficient of determination of at least $R^2 = .98$.

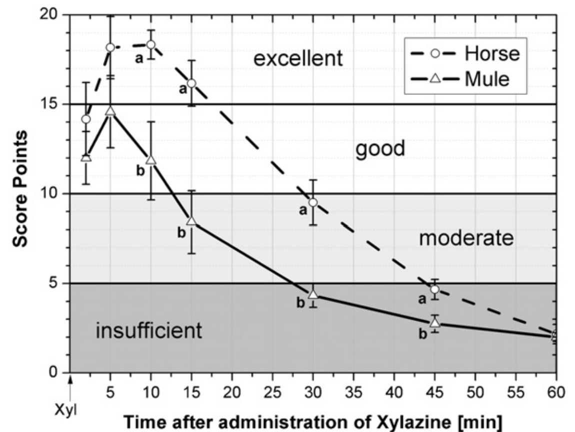

Fig. 4. Pharmacodynamics of xylazine. Resulting curves with summarized score points of behavior, standing ability, head height, drooping of the ear and of the lower lip, sensibility of the ear, and reaction to auditory and visual stimulus; a versus b ($P < .05$); Degree of sedation: 0-5 SPs = insufficient, >5-10 = moderate, >10-15 = good, >15-20 = excellent.

Figure 5 shows the plasma concentration versus time curve of xylazine for each of the three groups (H [n = 6], Mf [n = 8], and Mm [n = 6]). The two mule groups did not vary in a way that was statistically significant. Xylazine plasma concentration was much higher in the mules than in the horses. From 5 to 60 minutes after administration, mean xylazine plasma concentration in the horse group was significantly ($P < .05$) lower than that in each of the mule groups.

3.4. Results of Mathematical and Statistical Methods

Data analysis following the two-compartment open model had a correlation with the measured data of $R^2 = .99$. Significant ($P < .05$) differences between the M and the H group were found for β (velocity constant during elimination), B (relative y-intercept), k_{21} (velocity constant for distribution from peripheral to central compartment),

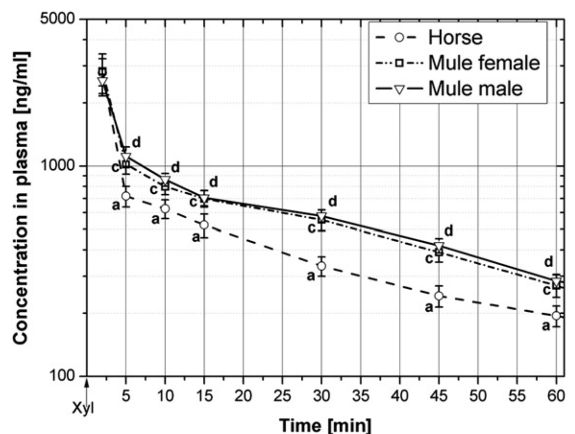

Fig. 5. Plasma concentration versus time curve; a versus c ($P < .05$), and a versus d ($P < .05$).

Table 2
Pharmacokinetic Data calculated after analysis using the two-compartment open model (PKAnalyst 1.0)

Value	Unit	Mule (n = 14)		Horse (n = 6)	
		Mean	Standard Deviation	Mean	Standard Deviation
α	min^{-1}	1.131	0.38	1.161	0.80
β	min^{-1}	0.023[a]	0.01	0.015[b]	0.00
$t_{1/2\alpha}$	minutes	0.705	0.33	1.648	2.27
$t_{1/2\beta}$	minutes	31.62[a]	8.63	46.58[b]	7.59
A	$ng \times min^{-1}$	22,245	13,880	37,061	20,412
B	$ng \times min^{-1}$	1,078.33[a]	287.23	635.07[b]	333.66
AUC	$ng \times min \times mL^{-1}$	64,836	16,621	65,512	33,568
AUMC	$ng \times min^{-2} \times mL^{-1}$	2,216,674	1,097,421	2,677,225	839,229
MRT	minutes	33.35[a]	8.46	45.00[b]	11.72
AUC_{trapez}	$ng \times minutes \times mL^{-1}$	73,699	21,286	96,611	60,913
$AUC_{(5-60)trapez}$	$ng \times minutes \times mL^{-1}$	72,091		42,238	
$AUC_{(0-tz)}$	$ng \times minutes \times mL^{-1}$[c]	62,148	15,804	63,472	34,849
$AUC_{(5-60)}$	$ng \times minutes \times mL^{-1}$	45,362		29,183	
$AUMC_{(0-tz)}$	$ng \times min^2 \times mL^{-1}$	1,710,411	745,098	2,210,124	1,113,581
$AUMC_{(5-60)}$	$ng \times min^2 \times mL^{-1}$	860,939		552,245	
$MRT_{(0-tz)}$	minutes	26.98[a]	5.49	36.23[b]	7.64
$AUC_{trapez(0-tz)}$	$ng \times minutes \times mL^{-1}$	71,713	21,149	94,238	61,995
$AUC_{trapez(5-60)}$	$ng \times minutes \times mL^{-1}$	56,964		35,283	
k_{12}	min^{-1}	0.720	0.27	0.677	0.48
k_{21}	min^{-1}	0.097[a]	0.06	0.043[b]	0.01
C_0	$ng \times mL^{-1}$	23,324	13,842	37,696	8,724
k_{el}	min^{-1}	0.338	0.16	0.456	0.36
$k_{el/2}$	min^{-1}	3.073	2.67	5.183	3.74
R^2	[d]	1.00	0.01	0.99	0.01

α, velocity constant during distribution; β, velocity constant during elimination; $t_{1/2\alpha}$, half-life during distribution; $t_{1/2\beta}$, half-life during elimination; A and B, extrapolated y-intercepts of distribution and elimination curve, respectively; AUC, area under the curve; AUMC, area under the moment curve; MRT, mean residence time; AUC_{trapez}, area under the plasma concentration curve, calculated using the trapezoidal method; $AUC_{(5-60)trapez}$, area under the plasma concentration curve, calculated using the trapezoidal method, with the values from 5 to 60 minutes; $AUC_{(0-tz)}$, area under the plasma concentration curve, calculated to the last measuring time point above limit of quantification; $AUC_{(5-60)}$, area under the plasma concentration curve, calculated using the values from 5 to 60 minutes; $AUMC_{(0-tz)}$, area under the moment curve to the last measuring time point above limit of quantification; $AUMC_{(5-60)}$, area under the moment curve, calculated using the values from 5 to 60 minutes; $MRT_{(0-tz)}$, mean residence time to the last measuring time point above limit of quantification; $AUC_{trapez(0-tz)}$, area under the plasma concentration curve, calculated using the trapezoidal method to the last measuring time point above limit of quantification; $AUC_{trapez(5-60)}$, area under the plasma concentration curve, calculated using the trapezoidal method between 5 and 60 minutes; k_{12}, velocity constant for distribution from central to peripheral compartment; k_{21}, velocity constant for distribution from peripheral to central compartment; C_0, extrapolated plasma concentration for xylazine at t_0; k_{el}, velocity constant for elimination processes from central compartment; $k_{el/2}$, velocity constant for half-life of elimination; R^2, coefficient of determination.

[a]versus[b]: $P < .05$.

[c]t_z, last time point of measurement for each individual.

[d]Coefficient of correlation for data to the two-compartment-model.

$t_{1/2\beta}$ (half-life during elimination), mean residence time (MRT), and $MRT_{(0-tz)}$ (residence time on last measuring time point above limit of quantification).

In the mules, pharmacokinetics of xylazine were as follows: $\beta \approx 0.023$/min, B $\approx$ 1,078 ng/min, $k_{21} \approx 0.097$/min, $t_{1/2\beta} \approx 32$ min, MRT $\approx$ 33 min, and $MRT_{(0-tz)} \approx 27$ min (refer Table 2 for details). Comparison of both groups shows that in the M group, β was 35% higher and extrapolated y-intercept B was 41% higher than in the H group. k_{21} was 55% higher in the M than in the H group. This resulted in a 15-minute shorter $t_{1/2\beta}$ and a 10- to 12-minute reduced $MRT_{(0-tz)}$ for xylazine in the mules.

4. Discussion

In the M group, sedation with 0.6 mg of xylazine per kilogram of body weight was shorter, less intense, and, in most cases, unsatisfactory. Sedation in the mules was good during the first 10 minutes, moderate at 15 minutes, and insufficient at 30 minutes. In most of the mules, sedation became unreliable between 10 and 15 minutes after the administration of xylazine, and floating of the teeth had to be stopped.

In the H group, sedation was excellent during the first 15 minutes, moderate at 30 minutes, and insufficient at 45 minutes. The mules recovered approximately 15 minutes faster from sedation than the horses. Mean degree of sedation in the mules was significantly ($P < .05$) lower at 10, 15, 30, and 45 minutes after xylazine administration. Mean xylazine plasma concentration was significantly ($P < .05$) higher in the mules at 5, 10, 15, 30, 45, and 60 minutes.

Velocity constant during the distribution of xylazine (α) in the vascular system was 1.13 minutes in the mules and 1.16 minutes in the horses. Half-life during the distribution ($t_{1/2\alpha}$) was 0.7 minutes in the mules and 1.7 minutes in the horses, and corresponded to the clinically evident significant alteration in heart beat and breathing rate minutes almost immediately after the administration of xylazine, which can be explained as a physiological reaction to xylazine-induced peripheral vasoconstriction mediated by α_2 receptors in the cardiovascular system. A significant difference was observed in k_{21} (0.097/min in the M group and 0.043/min in the H group), indicating a shorter effective period of xylazine in the peripheral compartment. The initial value for xylazine distribution immediately after intravenous bolus administration (V_d) was calculated for

each individual as follows: V_d = $dosage_{intravenous}/C_0$. The mean value for V_d was 48 mL/kg in the M group and 119 mL/kg in the H group. A small volume of distribution hints to a strong plasma protein binding and little assimilation in tissue. As a result, drug elimination is more efficient and therefore faster, because the V_d is smaller.

β was significantly higher in the mules (.023/min) than in the horses (0.015/min). The resulting elimination curve for xylazine in the mules was much steeper, which can be observed by a significant difference in the extrapolated y-intercept of the elimination curve B (1,078 ng/min in the mules compared with 635 ng/min in the horses). The resulting half-life during $t_{1/2\beta}$ was significantly lower in the mules (32 minutes) than in the horses (47 minutes), matching the results of Garcia-Villar et al. [20] (45 minutes), showing that xylazine elimination is far more effective in the mules than in the horses. At 30 minutes, that is, after the first $t_{1/2\beta}$, sedation in the mules was insufficient for clinical treatment and, at the same time, moderate in the horses. Sedation in the horses was insufficient at 45 minutes, which corresponds to the first $t_{1/2\beta}$.

The MRT of xylazine was 33 minutes in the mules and therefore significantly shorter than that in the horses (45 minutes). Similarly, residence time on last measuring time point above limit of quantification $MRT_{(0\text{-}tz)}$ was 27 minutes in the mules and thus significantly shorter than that in the horses (36 minutes).

The area under the plasma concentration curve (AUC) is calculated by integration of C(t) from zero to infinity and is proportional to the amount of drug that reaches the systemic blood system [22]. Values for the mules (a mean of 62.15×10^3 [ng $\times$ minutes $\times$ m^{-1}]) and values for the horses (a mean of 63.47×10^3 [ng $\times$ minutes $\times$ mL^{-1}) were almost congruent. However, $AUC_{(5\text{-}60)}$ in the mules in the first 60 minutes (45.36×10^3 [ng $\times$ minutes $\times$ mL^{-1}]) was 1.5 times higher than that in the horses (29.18×10^3 (ng $\times$ minutes $\times$ mL^{-1}]). In other words, during the first hour, xylazine concentration was higher in the central compartment in the mules than in the horses. Therefore, subspecies differences in the clinical effects of xylazine between mules and horses cannot be explained by means of the different amounts of plasma concentration, as a higher xylazine plasma concentration in the mules results in measurably less pronounced clinical effects. This is comparable with an interspecific study by Garcia-Villar et al. [20] in which horses had a 6-7 times higher plasma xylazine concentration than cattle when producing a similar behavioral effect.

The total clearance of a drug can be calculated independently of the pharmacokinetic model using equation (2) and (3).

$$CI_{tot} = K_{el} \times V_d \quad (2)$$

$$CI_{tot} = dosage_{iv}/AUC \quad (3)$$

This was calculated for each individual, with the same result in both formulas. Mean group values of total clearance were 9.87 mL/kg for the mules and 11.15 mL/kg for the horses.

The main causes for interspecies differences in PK and PD of drugs seem to be caused by variability in the physiological processes concerning drug adsorption, distribution, metabolism, and elimination and have been described for closely related species like *Equus caballus* (horse) and *Equus asinus* (donkey) as well as for other species [4].

Differences in pharmacokinetic parameters may, on the one hand, be caused by distinctions in physiology between mules and horses. Fluid balance and body water compartment partitioning in mules is more similar to that of the donkey and may be the cause for the differences in V_d, clearance, and $t_{1/2\beta}$ for xylazine in mules [11]. Besides, blood physiology in mules is slightly different [18,23], and a possibly idiosyncratic binding to plasma proteins or erythrocytes may affect the bioavailability of xylazine in mules.

Another explanation for the faster metabolism and elimination of xylazine in mules is that mules might have a different concentration, or activity, of the cytochrome P450 isoenzymes than horses or donkeys. According to Toutain et al. [4], the P450 cytochromes can vary considerably from species to species, with major differences between closely related species like horses and donkeys, or even in a given species (breed to breed). This variability seems to be due to both environmental and genetic factors.

To approximate sufficient sedation using intravenous administration of xylazine in the mules, several calculatory considerations were taken. Elimination of a drug can be estimated with 4-5 half-lives [22], which would be approximately 60 minutes for the mules and approximately 90 minutes for the horses. According to the two-compartment open model, $t_{1/2\beta}$ (and not $t_{1/2\alpha}$) was found to be the dominating half-life, which adds the most to the AUC. Mean $t_{1/2\beta}$ of the M group (32 minutes) increased by 50% ($\sim$15 minutes) would match the mean $t_{1/2\beta}$ of the H group (47 minutes).

As a fact, during a variety of clinical treatments in the researched livestock, reliable sedation in mules, comparable in duration and depth with a sedation using 0.6 mg of xylazine per kilogram of body weight intravenously in horses, was obtained with the administration of 0.9 mg of xylazine per kilogram of body weight intravenously to each mule (CP Bartmann, personal communication, 2007). Clinical perceptions of Matthews and Taylor [16] stating that mules apparently require approximately 50% more xylazine than horses underline this. However, a dose confirmation study would be needed to research whether or not pharmacokinetic data confirm these observations.

5. Conclusions

This study allowed gathering of combined results for level of sedation and pharmacodynamic and pharmacokinetic data under the influence of performing a dental treatment. The comparatively high number of 14 mules underlines the significance of the resulting data. Results of this study indicate significant differences in pharmacokinetics and pharmacodynamics of xylazine between mules and horses, supporting the findings of previous authors [4-7] that PK and PD of sedatives and anesthetics may vary significantly even between closely related species like horse, donkey, and mule. For xylazine, one of the most popular α_2 agonists in veterinary medicine, it could be shown that this dose of 0.6 mg/kg does not provide

adequate sedation for performing floating of teeth in mules. With the dose of 0.6 mg of xylazine per kilogram of body weight, mules recovered after 10 to 15 minutes after administration, which is approximately 15 minutes faster than horses (25-30 minutes). Sedation in the mules was less intense and, in most cases, unsatisfactory for floating of teeth.

Pharmacokinetic data for xylazine, calculated with the two-compartment open model, show that the dominating half-life $t_{1/2\beta}$, which is the half-life during elimination, is 15 minutes shorter in the mule (32 minutes) than in the horse (47 minutes). Several pharmacokinetic parameters of this study indicate that xylazine elimination is far more effective in the mule than in the horse.

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