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Pharmacokinetics and pharmacodynamic effects of tolazoline following intravenous administration to horses

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ABSTRACT

Tolazoline is an α_2-adrenergic receptor antagonist, used in veterinary medicine to antagonize the central nervous system depressant and cardiovascular effects of α_2 receptor agonists. The pharmacokinetics and pharmacodynamic effects of tolazoline when administered subsequent to detomidine in the horse were recently reported, although the reversal of the sedative and cardiovascular effects following detomidine may not be complete. The current study therefore investigated the pharmacokinetics and pharmacodynamic effects of tolazoline when administered as a sole agent. Nine healthy adult horses were administered tolazoline (4 mg/kg IV) and blood samples were collected at time 0 (prior to drug administration) and at various times up to 72 h post drug administration. Plasma samples were analyzed using liquid chromatography–mass spectrometry and resulting data analyzed using compartmental analysis.

Systemic clearance, steady state volume of distribution and terminal elimination half-life were 0.820 ± 0.182 L/h/kg, 1.68 ± 0.379 L/kg and 2.69 ± 0.212 h, respectively. Tolazoline administration had no effect on chin to ground distance, but the heart rate decreased (relative to baseline) and the percentage of atrial–ventricular block increased in all horses within 2 min of administration. Packed cell volume and glucose concentrations were also increased throughout the sampling period. While not commonly used as a sole agent, caution is indicated whenever tolazoline is administered since the effects may be unpredictable.

Introduction

Tolazoline is a non-selective α_2-adrenergic antagonist that is used both in human and veterinary medicine. In humans, tolazoline is commonly used to treat persistent pulmonary hypertension in infants (Nuntnarumit et al., 2002). In veterinary medicine, the drug is commonly used to reverse the sedative effects of α2-adrenergic agonists, especially in horses (Hubbell and Muir, 2006).

While it is currently only approved by the Food and Drug Administration (FDA) for the reversal of xylazine administration in horses, the α_2-adrenergic antagonistic effects of tolazoline following both xylazine and detomidine administration have been described (Kollias-Baker et al., 1993; Hubbell and Muir, 2006). While α2-adrenergic antagonists are beneficial in cases of overdoses of α2-adrenergic agonists, there are both anecdotal and documented reports of tachypnea, tachycardia, anxiety and death (Scofield et al., 2010). Following incremental intravenous (IV) dosing of tolazoline (average dose of 7.5 mg/kg) subsequent to xylazine, chin to ground distance increased significantly and the ventricular bradycardia and atrial–ventricular conduction disturbances were effectively reversed (Kollias-Baker et al., 1993). However, our laboratory has previously reported that that tolazoline (4 mg/kg IV) only transiently or incompletely antagonized the detomidine induced decrease in chin to ground distance, bradycardia and atrial–ventricular blocks (Knych et al., 2012). Interestingly, in the same study, packed cell volume (PCV) and glucose concentrations increased significantly with tolazoline administration (Knych et al., 2012). Similarly, Hubbell and Muir (2006) reported incomplete antagonism when tolazoline was administered subsequent to detomidine, but they did observe a shortened time to recovery in comparison to horses in a placebo control group.

Recently, we have studied the pharmacokinetics and some pharmacodynamic effects of tolazoline (4 mg/kg) when administered 1 h after sublingual detomidine (0.04 mg/kg) (Knych et al., 2012). In that study, the elimination half-life (3.77 h) of tolazoline was prolonged, relative to that described for detomidine and the clearance was relatively delayed. To the best of our knowledge, the only study describing the pharmacokinetics of IV tolazoline when administered as a sole agent to horses is the Freedom of

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Information summary (FOI) (Thurmon and Sisson, 1996) in which 1× and 5× the labeled dose of tolazoline (4.0 mg/kg and 20.0 mg/kg, respectively) were administered to ponies and blood samples collected up to 6 h post administration. The reported elimination half-lives were 1.0 and 1.7 h following administration of 4.0 mg/kg and 20.0 mg/kg tolazoline, respectively.

The difference in the reported half-life between the FOI study, when tolazoline alone was administered, and that reported by our laboratory (subsequent to detomidine), led us to investigate the pharmacokinetics of tolazoline administered as a sole agent. Additionally, since tolazoline may not completely reverse the sedative and cardiovascular effects of detomidine and (Hubbell and Muir, 2006; Knych et al., 2012), we also chose to investigate the effects of tolazoline on hematological (PCV, glucose), behavioral and cardiac parameters when it was administered as a sole agent.

Materials and methods

Animals

Six healthy, adult sedentary research horses were studied (three mares, three geldings; mean ± SD bodyweight [BW] 593 ± 55 kg; ages 10.5 ± 4.1 years). Food was withheld for 12 h prior and 12 h post drug administration. Water was available ad libitum throughout the course of the study. All horses were determined to be healthy and free of cardiovascular disease by physical examination, complete blood count, and a serum biochemistry panel that included aspartate aminotransferase, creatinine phosphokinase, alkaline phosphatase, total bilirubin, sorbitol dehydrogenase, blood urea nitrogen and creatinine. Horses were not given any other medications for a minimum of 2 weeks prior to this study.

This study was approved by the Institutional Animal Care and Use Committee of the University of California at Davis.

Instrumentation and drug administration

A 14 G catheter was aseptically placed in each external jugular vein. The right jugular vein catheter was used for drug administration, while the contralateral catheter was used for sample collection. Horses were weighed just prior to drug administration and each received a single IV dose of 4 mg/kg of tolazoline (Telazol, Lloyd Laboratories). Following administration, the catheter was flushed with 10 mL of a dilute heparinized saline solution (10 IU/mL). Subsequent to dosing the right jugular vein catheter was removed.

Sample collection

Blood samples were collected at the following times: 0 (prior to drug administration), 5, 10, 15, 30, and 45 min and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 18, 24, 48 and 72 h post tolazoline administration. Prior to drawing each sample of blood for analysis of drug concentrations, 10 mL of blood was aspirated and discarded from the catheter and T-Port extension set (combined internal volume <2 mL). The catheter was flushed with 10 mL of a dilute heparinized saline solution (10 IU/mL) following each sampling time. The left jugular vein catheter, used for sample collection, was removed following the 8 h sample collection and the remaining samples collected via direct venepuncture. Blood samples were collected into EDTA blood tubes (Kendall/Tyco Healthcare) and stored on ice until centrifugation at 3000 *g* for 10 min at −4 °C. Plasma was then immediately transferred into storage cryovials (Phenix Research Products) and stored at −20 °C until analysis (approximately 2 weeks following collection of the final sample).

Additional samples were collected for plasma glucose, PCV and plasma protein (PP) analysis via the IV catheter. For blood glucose concentration determination, samples were collected at −24 h (1 day prior to drug administration), 0 (immediately prior to drug administration), 15, 30, and 45 min and 1, 1.5, 2, 2.5, 3, 4, 5, and 6 h post tolazoline administration. Glucose samples were collected into sodium heparin blood tubes (Kendall/Tyco Healthcare). Samples were analyzed by the Clinical Pathology Laboratory of the William R. Pritchard Veterinary Medical Teaching Hospital of the University of California, Davis, using the laboratory's standard protocol for glucose analysis. Samples for analysis of PCV and PP were collected at 0 (immediately prior to tolazoline administration), 5, 10, 15, 30 and 45 min and 1, 1.5, 2, 2.5, 3, 4, and 6 h post tolazoline administration. PCV and PP samples were collected into EDTA blood tubes (Kendall/Tyco Healthcare). PCV was measured via microhematocrit and PP via refractometer.

Determination of plasma tolazoline concentrations

The analytical reference standard for tolazoline was obtained from Sigma–Aldrich. The internal standard (IS) medetomidine was obtained from Orion Corporation. Stock solutions of tolazoline were prepared at 1 mg/mL in methanol. Quantitative analysis was performed on a triple quadrupole mass spectrometer (TSQ Quantum Ultra, Thermo Scientific) equipped with a liquid chromatography system (Model 1100, Agilent Technologies). Chromatography employed an Eclipse XDB-Phenyl, 150 mm × 3 mm, 3.5 μm, column (Agilent Technologies) and a linear gradient of acetonitrile (ACN) in water with a constant 0.2% formic acid at a flow rate of 0.45 mL/min. The initial ACN concentration was held at 1.0% for 0.4 min, and ramped up to 95% over 4.6 min. The ACN concentration was held at 95% for 0.5 min and then the column was re-equilibrated at initial conditions for 2.8 min.

Tolazoline working solutions were prepared by dilution of the stock solution with methanol to concentrations of 0.01, 0.1, 1 and 10 ng/μL. Plasma calibrators were prepared by dilution of the working standard solutions with drug free plasma to concentrations of 0.5, 1, 2.5, 5, 10, 25, 50, 100, 200, 350, 500, 750 and 1000 ng/mL. All plasma calibration curves used quadratic equation as best fit with a $1/X$ weighting. All curves gave correlation coefficients of (R^2) of 0.99 or better. Calibration curves and negative control samples were prepared fresh for each quantitative assay. To evaluate the accuracy and precision of the analytical method, quality control samples were prepared by spiking blank plasma samples with tolazoline at concentrations of 3.0 and 350 ng/mL. The calibrators and the quality control samples were treated in the same way as the samples. Prior to analysis, plasma samples, calibrators and quality control sample were allowed to thaw at room temperature. An initial sample volume of 0.5 mL plasma had plasma proteins precipitated with the addition of 0.5 mL 9:1 ACN:1 M acetic acid containing the IS (20 ng/mL). All samples were vortex mixed, refrigerated, and vortex mixed for a second time, followed by centrifugation (3830 *g* at 4 °C). The injection volumes were 10.0 μL.

Detection and quantification employed selective reaction monitoring of initial precursor ion for tolazoline (mass to charge ratio (m/z) 161.1). The response for the major product ions for tolazoline (m/z 65.1, 91.1) were plotted and peaks at the proper retention time integrated using Quanbrowser software, Thermo Scientific). The IS (mass to charge ratio (m/z) 201.1) product ion response was monitored (m/z 68.1, 95.1).

The concentration of tolazoline was determined by an IS method. The technique was optimized to provide a limit of detection (LOD) of 0.1 ng/mL and limits of quantitation (LOQ) of 0.5 ng/mL for tolazoline.

Pharmacokinetic analysis

Compartmental analysis was used for determination of pharmacokinetic parameters for IV administered tolazoline using commercially available software (Phoenix WinNonlin version 6.2). Only data points (plasma concentrations) equal to or above the LOQ for the assays were included in the analysis. Based on coefficient of variation, Akaike Information Criterion and visual inspection of the residual plots, a two-compartment model ($C_p = Ae^{-\alpha t} + Be^{-\beta t}$) with a weighting factor of $1/\hat{y}$ gave the best fit to concentration data points from individual animals. The area under the curve (AUC) and area under the moment curve (AUMC) were extrapolated to infinity using the last measured plasma concentration divided by the terminal slope λ_z.

Physiologic parameters

Heart rate and rhythm parameters were collected for a minimum of 30 min prior to and at 0, 2, 5, 8, 10, 12, 15, 20, 30 and 45 min, 1, 1.5, 2, 2.5, 3, 4, 5 and 6 h post drug administration. Each horse was equipped with a Holter monitor (Forrest Medical) for assessment of cardiac effects. Heart rate was evaluated at predetermined time points via manual counting of P-QRS-T complexes over a 1 min time period. The percentage of atrial signals blocked by the atrial–ventricular node pre and post tolazoline administration was calculated using the formula (atrial rate–ventricular rate)/atrial rate. Atrial and ventricular rates were determined by manually counting P waves and P-QRS-T complexes, respectively, over a 1 min period at 0, 2, 5, 8, 10, 12, 15, 20, 30 and 45 min, 1, 1.5, 2, 2.5, 3, 4, 5 and 6 h post drug administration.

As an indirect measure of sedation or excitation, chin to ground distance was also recorded for each horse. A paper measuring tape was used to measure the distance from the animals muzzle to the ground at 0, 5, 10, 15, 30 and 45 min, 1, 1.5, 2, 2.5, 3, 4, 5, 6, and 8 h post drug administration.

Gastrointestinal borborygmi were recorded, by the same individual, via direct auscultation with a stethoscope at 30 min prior to and at 0 (immediately prior to drug administration), 5, 10, 15, 30 and 45 min, 1, 1.5, 2, 2.5, 3, 4, 5, 6 and 8 h post drug administration. All four quadrants were auscultated and sounds recorded as normal, increased, decreased or absent relative to baseline. Incidence of defecation as well as consistency of stool was observed and recorded throughout the sampling period. Any additional physiological or behavioral observations were noted as observed throughout the study.

Statistical analysis

Statistical analyses using commercially available software (SAS) were performed to assess significant differences in physiologic variables, PCV, TP and glucose values both pre and post reversal agent administration for individual horses. Raw data for all physiological variables as well as glucose, PCV and TP were checked