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Effect of Intravenous Administration of Cobalt Chloride to Horses on Clinical and Hemodynamic Variables

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Background: Cobalt chloride ($CoCl_2$) is administered to racehorses to enhance performance. The purpose of this study was to evaluate the clinical, cardiovascular, and endocrine effects of parenterally administered $CoCl_2$.

Objectives: To describe the effects of weekly intravenous doses of $CoCl_2$ on Standardbred horses.

Animals: Five, healthy Standardbred mares.

Methods: Prospective, randomized, experimental dose-escalation pilot. Five Standardbred mares were assigned to receive 1 of 5 doses of $CoCl_2$ (4, 2, 1, 0.5, or 0.25 mg/kg) weekly IV for 5 weeks. Physical examination, blood pressure, cardiac output, and electrocardiography (ECG) were evaluated for 4 hours after administration of the first and fifth doses. Blood and urine samples were collected for evaluation of cobalt concentration, CBC and clinical chemistry, and hormone concentrations.

Results: All mares displayed pawing, nostril flaring, muscle tremors, and straining after $CoCl_2$ infusion. Mares receiving 4, 2, or 1 mg/kg doses developed tachycardia after dosing (HR 60–126 bpm). Ventricular tachycardia was noted for 10 minutes after administration of the 4 mg/kg dose. Increases in systolic arterial pressure (SAP), diastolic arterial pressure (DAP), and mean arterial pressure (MAP) occurred after administration of all doses (4, 2, 1, 0.5, and 0.25 mg/kg). Profound hypertension was observed after the 4 mg/kg dose (SAP/DAP, MAP [mmHg] = 291–300/163–213, 218–279). Hemodynamics normalized by 1–2 hours after administration. ACTH and cortisol concentrations increased within 30 minutes of administration of all $CoCl_2$ doses, and cardiac troponin I concentration increased after administration of the 4 and 2 mg/kg doses.

Conclusions and Clinical Importance: The degree of hypertension and arrhythmia observed after IV $CoCl_2$ administration raises animal welfare and human safety concerns.

Key words: Blood doping; Cobalt; EPO; Hematuria; Hypertension; Pharmacokinetics; Racehorses.

Cobalt is an essential micronutrient that is present in mammalian systems in organic and inorganic (ionic) forms. Importantly, cobalt ion is a central cofactor of vitamin B12 (cobalamin) and is required for proper nucleotide synthesis, fatty acid metabolism, neural function, and hematopoiesis, among other functions.[1] While regular intake of trace amounts of dietary cobalt is required for health, overt clinical disease associated with acute or long-term dietary deficiency has not been reported in horses (even when horses are grazing pastures that have been associated with cobalt deficiency in ruminants); this is likely a result of adequate synthesis of vitamin B12 in the equine gastrointestinal tract.[2,3] Dietary cobalt requirements for horses undergoing heavy exercise have been estimated at 0.5–0.6 mg per horse per day,[2] which is a miniscule amount compared to that present in some cobalt-containing supplements or that given as cobalt salts to horses to enhance performance.

Abbreviations:

AUC	area under the curve
CO	cardiac output
cTnI	cardiac troponin I
DAP	diastolic arterial pressure
ECG	electrocardiography/electrocardiogram
EPO	erythropoietin
ICP-MS	inductively coupled plasma mass spectrometry
MAP	mean arterial pressure
SAP	systolic arterial pressure

Aside from its pleiotropic roles in intermediary metabolism, cobalt as inorganic cobalt salts is also an effective hypoxia mimetic,[1,3] and this characteristic has been extensively exploited both for valid reasons (therapy for anemia; facilitation of laboratory investigations of hypoxia on various biological systems) and for illicit use (to enhance performance in elite human athletes).[1,4–6] In human athletes, cobalt administered at pharmacologic doses is associated with increased erythropoietin (EPO) synthesis and increased red blood cell mass, which is thought to confer competitive advantage particularly in endurance competitions, where this practice represents a form of blood doping.[1,7,8] In both professional and amateur sports, this is a violation of fair competition, as well as regulations of governing bodies of specific sporting disciplines and has been treated as such by regulatory bodies.[9,10] Cobalt is a prohibited

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substance in sports under the hypoxia-inducible factor (HIF) stabilizers category of the World Anti-Doping Agency.[10]

Recently, anecdotal and regulatory reports of cobalt chloride ($CoCl_2$) administration to Thoroughbred and Standardbred racehorses as a performance-enhancing substance have surfaced in several racing jurisdictions around the world, including the United States, Australia, Hong Kong, and Canada.[3,7,11–13] In addition, anecdotal reports of complications and sudden death after intravenous administration of $CoCl_2$ are circulating among horsemen and regulatory officials within the racing industry, raising a major issue concerning animal welfare within this industry. While single-dose pharmacokinetics of $CoCl_2$ in adult horses have been reported[14] and have provided guidance for setting preliminary regulatory thresholds in postrace samples from racing animals in some jurisdictions, the effects of $CoCl_2$ infusion on the cardiovascular physiology of horses, including toxicosis, have not been reported in detail. Furthermore, chronic cobalt exposure has been associated with neurotoxicosis, cardiotoxicosis, and endocrine abnormalities in humans primarily in association with occupational exposure and cobalt-containing orthopedic implants.[1,15] Racehorses are reportedly dosed with $CoCl_2$ repeatedly, but information regarding multiple-dose pharmacokinetics and the pharmacodynamic effects of chronic exposure of horses to this substance is scarce. Based on the suspected frequency of its illicit use in equine racing populations, additional data regarding the distribution and biological effects of $CoCl_2$ in horses are needed to inform regulatory policy and proper medical care of the animals. Therefore, the goal of this study was to describe the physiological and biochemical (including endocrine) effects of weekly intravenous administration of different doses of $CoCl_2$ to healthy Standardbred horses, particularly those related to cardiovascular physiology that might affect racing performance. In addition, this study evaluated the cumulative pharmacokinetics of weekly dosing of $CoCl_2$ (n = 5) to horses.

Materials and Methods

Experimental Design

All experimental procedures were approved by The Ohio State University Institutional Animal Care and Use Committee in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Five untrained, healthy, adult Standardbred mares owned by The OSU College of Veterinary Medicine and housed at the College teaching and research farm were studied in a prospective, randomized experimental study. Mares were 12–13 years of age, weighed 460–530 kg, and were considered healthy based on physical examination and routine bloodwork (CBC, serum biochemistry). During the study, mares were stall-confined with free-choice access to grass hay and water. Each mare was randomly assigned to receive 1 of 5 doses of $CoCl_2$ (4, 2, 1, 0.5, or 0.25 mg/kg) as an intravenous bolus (infused over 1 minute) once weekly for 5 weeks; each individual horse received the same assigned dose during each week of the study. Cobalt chloride was prepared by a compounding pharmacy[a] as a 200 mg/mL solution dissolved in water with 1% benzyl alcohol as a preservative for intravenous infusion and was administered undiluted. The concentration of cobalt in this solution was not analyzed.

Instrumentation, Sample Collection, and Sample Analysis

Before each dose, animals were instrumented with pulmonary artery[b] and right atrial[c] catheters for evaluation of blood pressure and cardiac output, a transverse facial artery catheter[d] for evaluation of blood pressure, 2 external jugular venous catheters[e] (1 assigned for drug administration and 1 for blood sampling), an indwelling urinary catheter and continuous urine collection system,[f] and electrocardiography leads.[g] Blood was collected at baseline and at 5, 10, 15, 20, 30, 45, 60, 120, and 240 minutes after each cobalt dose; additional blood samples were collected at 24, 48, 72, 96, 120, and 144 hours postdose. Urine was collected at baseline and 15, 30, 60, 120, and 240 minutes after each cobalt dose; additional urine samples were collected at 24, 48, 72, 96, 120, and 144 hours postdose. Physical examination data (heart rate, respiratory rate, rectal temperature, mucous membrane color, capillary refill time, digital pulse character) were collected at baseline and at 5, 10, 15, 20, 30, 45, 60, 120, and 240 minutes postdosing, as well as at 24, 48, 72, 96, 120, and 144 hours postdose. Discomfort was subjectively assessed by observations of behavior, posture, muscle tremors, straining, and signs of abdominal pain. Transabdominal ultrasonography[h] of the spleen was performed during and immediately after $CoCl_2$ administration on week 2 to assess splenic contraction. Blood pressure (systolic arterial pressure [SAP], diastolic arterial pressure [DAP], and mean arterial pressure [MAP]), electrocardiography (ECG), and cardiac output (CO) data (thermodilution)[i] were collected at baseline and at 5, 10, 15, 20, 30, 45, 60, 120, and 240 minutes postdosing. All catheters were removed from the horses after the 240 minute time point. Blood samples were collected into serum clot, heparin-containing, and EDTA-containing tubes and centrifuged at 2,000×*g* for 10 minutes at 4°C within 4 hours of collection; serum, plasma, and urine samples were aliquoted and stored at −80°C until analysis. Plasma samples were analyzed within 2 months of collection, and urine samples were analyzed within 4 months of collection.

Blood and Urine Analysis

Hematocrit, red blood cell count, white blood cell count, platelet count, plasma protein, serum electrolytes, markers of renal and liver function, and blood gas analyses (including L-lactate) were evaluated frequently over the first 24 hours after $CoCl_2$ administration; these analyses were performed with the hematology and chemistry analyzers located in an accredited veterinary diagnostic laboratory. Urine was collected constantly and sampled periodically over 4 hours after $CoCl_2$ administration, grossly evaluated for evidence of abnormalities consistent with urinary tract damage, and submitted for urinalysis. Individual urine samples were obtained at the designated time points through aspiration of urine from the indwelling catheter with a syringe.

Cobalt Analysis

Plasma and urine cobalt concentrations were measured with an Agilent 7500cx inductively coupled plasma mass spectrometry (ICP-MS) instrument, ASX-500 series autosampler, and Agilent MassHunter software version B.01.01 at the Ohio Department of Agriculture Analytical Toxicology (ATL) Section of the Consumer Protection Laboratory (CPL) (ISO 17025; A2LA certification). The ICP-MS was tuned daily before sample analysis with Agilent 7500 ICP-MS tuning solution (p/n 5185-5959). Samples were

diluted directly into a solution containing 0.5% (w/v) nitric acid,[j] 2% (v/v) isopropyl alcohol,[k] and 0.05% Triton X-100. Cobalt in the study samples was measured at a m/z of 59, and the samples were infused alongside a 100 ppb germanium internal standard solution that was measured at a m/z of 72. Cobalt concentrations were determined with a linear regression calibration curve prepared from cobalt standard solutions and verified against positive controls prepared from a NIST 1640a solution. Individual runs were deemed acceptable when the R^2 of the calibration curve was greater than 0.99, positive controls were within 20% of their nominal value, and the RSD of the intra-assay variation was <20% when determined from continuing calibration verification samples. The method limit of quantification was 0.15 ng/mL, and the same-day and between-day characteristics of the assay are provided (Table 1).

Pharmacokinetic Analysis

Multiple-dose plasma concentration versus time data after administration of the 5 doses until the last sample time point, for each horse (each given a different dose of elemental cobalt), was analyzed by noncompartmental analysis by commercially available software.[l] The area under the curve and area under the moment curve were estimated by the log up-linear down trapezoidal method and extrapolated to infinity using the final serum concentration >LOQ divided by the terminal slope (λ_z; calculated from at least 3 of the terminal data points). Once all of the initial parameters were calculated, the data from each horse/dose from all 5 doses were re-analyzed simultaneously to calculate the average plasma cobalt concentration, the percentage fluctuation between C_0 and C_{min}, clearance at steady state, and accumulation factor. AUC/τ was calculated by dividing the total AUC by the dose interval (168 hours). Average plasma concentration was calculated by relationship among the dose, plasma clearance, and dose interval:

$$C_{avg} = \frac{\text{Dose}}{\text{Cl}_t \cdot \tau}$$

where C_{avg} is the average plasma concentration of cobalt, Dose is the dose (mg/kg) each horse received at the interval t, multiplied by the total clearance (Cl_t). The percentage fluctuation in plasma concentration was the ratio of peak cobalt: trough cobalt concentrations expressed as a percentage. The accumulation factor was determined using the following equation:

$$\text{Accumulation Factor} = \frac{(1 - e^{-n \cdot \lambda_z \cdot \tau})}{(1 - e^{-\lambda_z \cdot \tau})}$$

where e is the base of the natural logarithm; n = the number of doses administered; λ_z is the terminal rate constant; and τ is the dose interval (hours). Data are summarized by dose (Table 2). Urine concentrations of cobalt were analyzed by noncompartmental analysis to determine the rate constant of the terminal phase, half-life of terminal phase, maximum urine concentration, time of maximum concentration, the area under the curve to the last measured time point, AUC extrapolated to infinity, and clearance for the first and last cobalt dosing intervals. The AUC was calculated by the log-linear trapezoidal rule, and clearance was calculated by dividing total dose administered during that interval by the AUC for the 1st and 5th dosing intervals.

Table 1. Performance characteristics of the inductively coupled plasma mass spectrometry assay used to measure plasma cobalt concentration in this study.

Theoretical Concentration (ng/mL; ppb)	Measured Concentration ± 95% CI (ng/mL; ppb)	Accuracy (%)
Same day		
0.59	0.601 ± 0.007	102 ± 0.5
5.9	5.54 ± 0.2	94 ± 2
Between day		
0.59	0.59 ± 0.03	98.7 ± 2
5.9	5.54 ± 0.2	93 ± 1

Cardiac Troponin I and Hormone Analysis

Serum cardiac troponin I (cTnI) concentrations were measured with an immunoassay system.[16,17,m] Serum erythropoietin concentrations were measured with an equine-specific ELISA.[14,n] Serum cortisol, insulin, and thyroid hormone concentrations were measured with immunoassays.[o] Plasma ACTH concentrations were measured with an immunometric assay.[p] Serum biochemistry analyses were performed on a clinical chemistry analyzer.[q] Ionized calcium, ionized magnesium, and blood gas analyses were performed with a chemistry analyzer.[r]

Hormones were measured multiple times every week, before and after $CoCl_2$ administration. A total of 70 selected samples from all horses, collected over a 2-month period (0 minutes, 2, 6, 12, 24, and 48 hours, 2 months), were evaluated for serum EPO concentrations. Thyroid hormones (T3, T4) were measured at 0 minutes, 1, 2, 12, 24, and 72 hours, and 2 months; insulin and ACTH concentrations were measured at 0, 30 minutes, 1, 2, 6, 12, 24, and 72 hours.

Results

Clinical/behavioral Assessment

All mares were anxious after receiving the infusion, showing nostril flaring, muscular tremors and fasciculation, pawing, and straining to urinate by 5 minutes after the $CoCl_2$ infusion; this persisted for ~60 minutes in mares receiving higher doses (4, 2, and 1 mg/kg). Mares receiving higher doses (4, 2, and 1 mg/kg) also displayed mild-to-moderate signs of abdominal pain in

Table 2. Change in hematocrit after cobalt chloride administration to healthy Standardbred horses. Panel A represents the change in hematocrit observed in the 2 hours after infusion. Panel B represents the change in baseline hematocrit after serial (n = 5) dosing over a period of 5 weeks.

(A)

Dose	Baseline	5 min	15 min	30 min	60 min	120 min
4 mg/kg	40.8	62.2	53.1	53.1	45.5	40.3
2 mg/kg	37.2	54.5	43	39.8	37	36.3
1 mg/kg	41.4	63.2	41.1	40.6	36.6	36.5
0.5 mg/kg	34	42.4	37.5	34.2	32.9	34.1
0.25 mg/kg	37.5	40	37.3	36.3	37.9	39.5

(B)

Dose	Week 1	Week 2	Week 3	Week 4	Week 5
4 mg/kg	40.8	42	42	41.7	41.7
2 mg/kg	37.2	37.2	41.7	37.6	36
1 mg/kg	41.4	39	38	42	38.9
0.5 mg/kg	34	33.7	34.1	33	34.6
0.25 mg/kg	37.5	38.8	37	38.8	39

the 15–20 minutes after drug infusion evidenced by treading, kicking at abdomen, posturing repeatedly to urinate.

Cardiovascular Variables

Mares receiving the higher $CoCl_2$ doses (4, 2, and 1 mg/kg) developed tachycardia within 1 minute after drug infusion (Fig 1), but this was not observed in animals receiving lower doses (0.5 and 0.25 mg/kg). Cardiac dysrhythmias (including short paroxysms of ventricular tachycardia) occurred in the first 10 minutes after administration on all 5 administrations to the mare receiving the 4 mg/kg dose. Increases in SAP, DAP, and MAP occurred at all doses, while profound hypertension was observed in the horse receiving the 4 mg/kg dose (SAP/DAP, MAP [mmHg] = 291–300/163–213, 218–279). Cardiac output increased after administration of all doses but more than doubled in mares receiving the highest doses, returning to baseline values between 45 and 60 minutes after $CoCl_2$ infusion. Mares receiving the 4 and 2 mg/kg doses developed prominent oral mucous membrane congestion that persisted for ~20 minutes after dosing and subsequently resolved. At all doses, cardiovascular variables returned to baseline by 1–2 hours after administration.

Blood Variables

A transient increase in hematocrit and red cell count occurred in mares receiving the highest $CoCl_2$ doses, with these values returning to baseline levels within 1 hour of drug administration. The increase in hematocrit was associated with splenic contraction observed ultrasonographically. During (5 weeks) and after (2 months) cobalt administration, changes in hematological variables were not evident for any of the mares. No evidence of induced hematopoiesis (sustained increase in baseline hematocrit) was observed in any of the treated horses (Table 2). No major variations in electrolyte concentrations were noted. Mild increases in L-lactate and glucose concentrations occurred with the highest $CoCl_2$ doses but returned to baseline concentrations within 1 hour.

Urinary Variables

Urine from horses receiving the highest doses became discolored (red to red-brown), tested positive for blood on a urine dipstick, and contained visible tissue debris as early as 15 minutes after infusion; these gross changes persisted for up to 240 minutes after $CoCl_2$ administration (Figs 2 and 3). Urine sediment contained large numbers of urinary epithelial cells and erythrocytes after administration of $CoCl_2$.

Erythropoietin, Insulin, ACTH, Cortisol

No change in [EPO] within or between horses was observed during the study. Similarly, no detectable change in concentrations of thyroid hormones or insulin was observed. All mares showed increases in serum cortisol concentrations in the 4–6 hours after cobalt administration, and all mares but the one receiving the 0.25 mg/kg dose had an increase in plasma ACTH concentration during the same time frame (Fig 4).

Cardiac Troponin I

No change in the serum concentration of cTnI was observed in mares receiving lower doses of $CoCl_2$ (1,

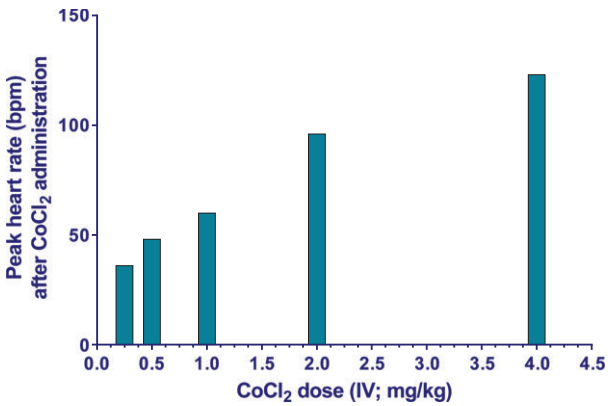

Fig 1. Effects of infusion of various doses of $CoCl_2$ on heart rate of healthy Standardbred mares. Each bar represents the peak heart rate response of an individual mare receiving a single intravenous dose of $CoCl_2$ (4, 2, 1, 0.5, or 0.25 mg/kg) during the 15–20 minute period after infusion (first dose of 5). Mares receiving higher doses (4, 2, and 1 mg/kg) developed tachycardia after $CoCl_2$ administration.

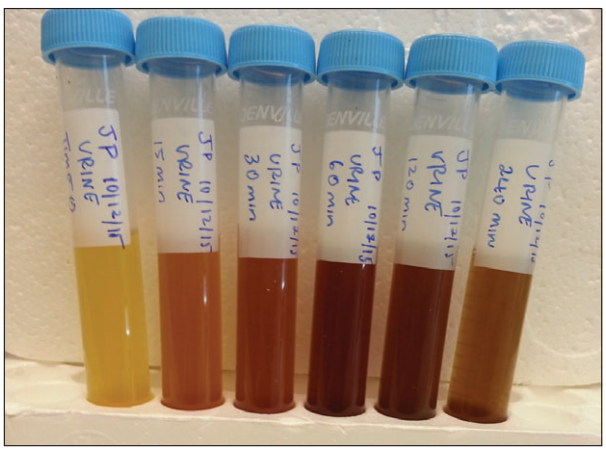

Fig 2. Gross change in urine color after intravenous administration of $CoCl_2$ (4 mg/kg) to a healthy mare. A baseline (predosing) urine sample is displayed on the far left, followed by samples collected at 15, 30, 60, 120, and 240 minutes postdosing (from left to right). Urine discoloration was evident as early as 15 minutes postdosing and persisted for at least 240 minutes after $CoCl_2$ administration.

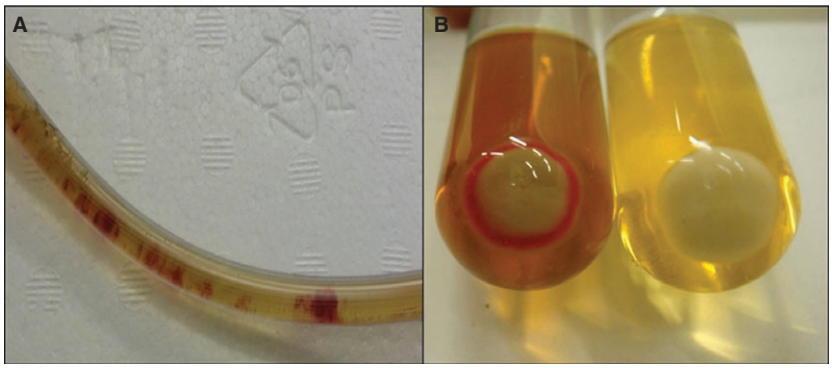

Fig 3. Changes in the gross appearance of the urine after intravenous administration of $CoCl_2$ (4 mg/kg) to a healthy mare. Tissue debris was present within the urinary collection system shortly after administration (**A**), and centrifuged urine and urine sediment (**B**, left = post-$CoCl_2$; right = baseline) were discolored. The urine sediment contained large numbers of urinary epithelial cells and erythrocytes post-administration.

0.5, and 0.25 mg/kg) at any time point. Mares receiving the 4 and 2 mg/kg doses had increased serum cTnI concentrations within 2 hours of dosing, with peak concentrations (0.24 and 0.05 ng/mL, respectively, after the first infusion; reference range <0.01 ng/mL) developing by 6–12 hours after infusion and returning to baseline by approximately 24–48 hours postinfusion. Peak cTnI concentrations measured in the mare receiving the 4 mg/kg dose were 0.24 ng/mL after the first infusion, 0.13 ng/mL after the third infusion, and 0.08 ng/mL after the fifth infusion (Fig 5).

Plasma and Urinary Cobalt Concentrations

Baseline plasma cobalt concentrations in these horses before administration of the first dose of $CoCl_2$ were lower (3.6 ± 3.1 ppb) than the regulatory tolerance value (25 ppb) established by the Ohio State Racing Commission for postrace blood samples.[18] Select pharmacokinetic variables associated with serial intravenous administration of CoCl2 at the dosages used in this study are presented in Table 3. The maximum plasma concentration of cobalt observed after the 1st–5th doses of $CoCl_2$ at the 4 mg/kg dose was 10,036; 10,457; 11,719; 14,535; and 17,782 ng/mL (ppb), demonstrating accumulation of cobalt after each injection. Similar, albeit lower, plasma concentrations were observed after the 2, 1, 0.5, and 0.25 mg/kg doses in each of those horses (Fig 6). Likewise, trough concentrations (immediately before the subsequent doses) increased with drug dose and number of doses. After the first dose in the horse receiving the 4 mg/kg dose, plasma cobalt trough concentrations were 738 ng/mL, increasing to 1,597 ng/mL 168 hours after the 5th dose. Accumulation factors after 5 doses were 2.6–3.3-fold higher than the 1st dose (0.25–4 mg/kg). The geometric mean (range) of plasma half-life of cobalt for all horses in this study was 12 days (9.8–13.6 days). Cobalt accumulation in horses receiving 4 and 2 mg/kg was associated with reduced clearance compared to horses receiving 1, 0.5, and 0.25 mg/kg doses after the 5th dose. Similarly, the V_z and V_{ss} in horses receiving the 4 and 2 mg/kg doses

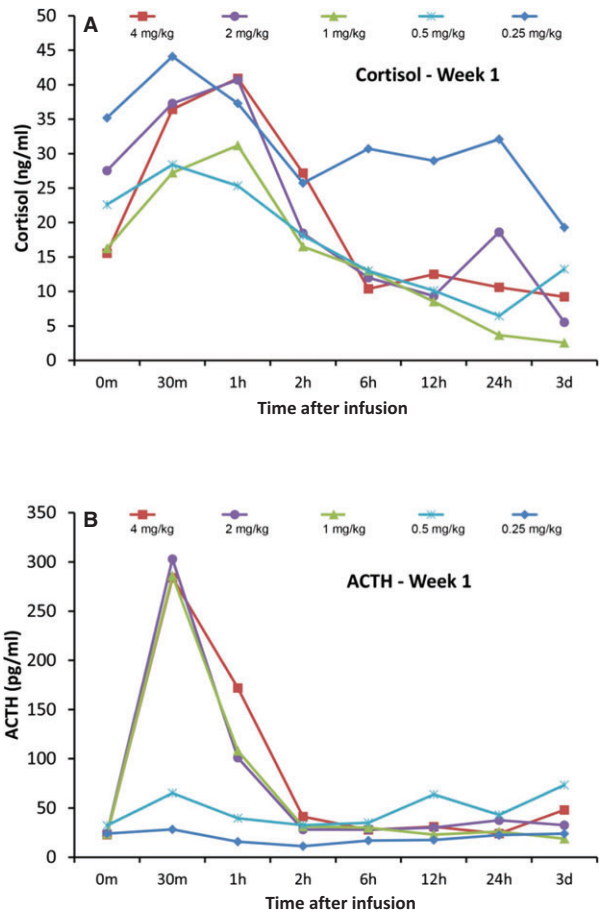

Fig 4. Serum cortisol (panel **A**) and plasma ACTH (panel **B**) concentrations in healthy horses after the first ("Week 1") of 5 intravenous administrations of $CoCl_2$ (4, 2, 1, 0.5, and 0.25 mg/kg). Hypothalamic-pituitary-adrenal axis activation occurred after all $CoCl_2$ doses. Doses of 4, 2, and 1 mg/kg elicited the highest ACTH responses.

were lower than those horses receiving ≤1 mg/kg after the 5th dose. The fluctuation in plasma concentrations from peak to trough declined between the 1st and 5th

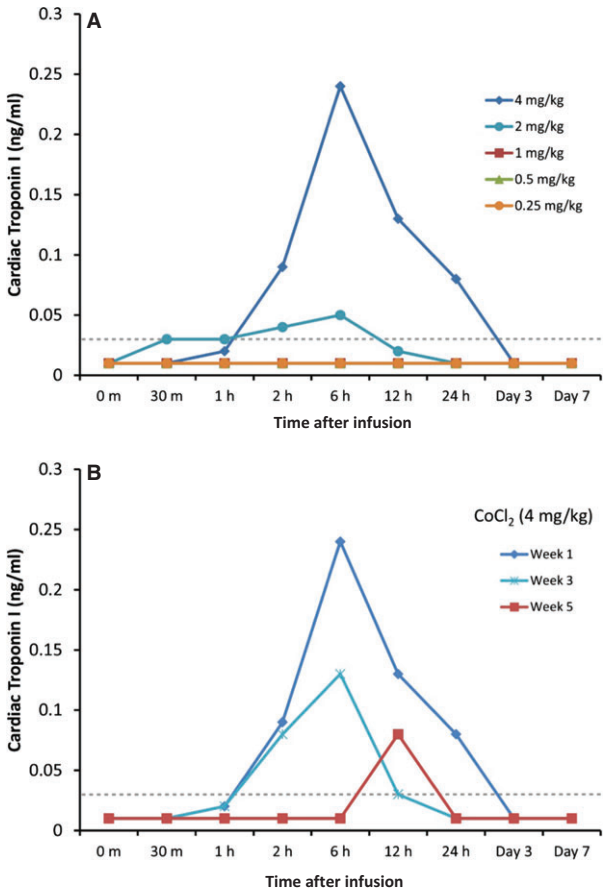

Fig 5. Serum cardiac troponin I concentrations in healthy horses after intravenous administration of $CoCl_2$ (4, 2, 1, 0.5, and 0.25 mg/kg). Panel **A** displays results from all horses from week 1 (after the first of 5 total doses); Panel **B** displays results of the horse administered the highest $CoCl_2$ dose (4 mg/kg) from weeks 1, 2, and 3. Peak cTnI concentrations were noted within ~6 hours of dosing, returning to baseline by 1–3 days after dosing. There is evidence for attenuation of the magnitude of this effect over time (Panel **B**). The dashed line represents the upper limit of the normal reference range for troponin I in adult horses.

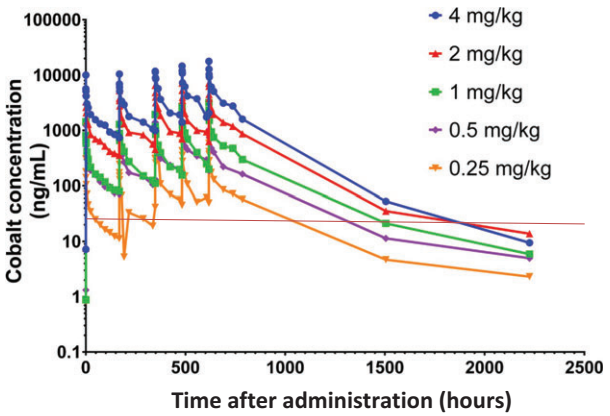

Fig 6. Plasma concentration-time profiles after intravenous administration of multiple doses of $CoCl_2$ at varying dosages (4, 2, 1, 0.5, or 0.25 mg/kg) to 5 Standardbred mares. C_{min} concentrations after the final dose were higher than the samples collected before dosing for all horses, which shows accumulation of $CoCl_2$ with repeated dosing at weekly intervals. Fluctuation of the peak and trough concentrations differed by dose (actual C_{max}, C_{min}; fluctuation % = 643–1,183%). The thin red horizontal line indicates the actionable regulatory threshold for plasma cobalt concentrations of 25 ng/mL currently used by the Ohio Racing Commission for evaluation of postrace samples.

doses at the cobalt doses of ≥2 mg/kg in comparison with the lower doses administered. After the 5th dose, the percentage fluctuation was 643–686% at the 4 and 2 mg/kg doses but increased from 849 to 1,183% as doses decreased from 0.5 to 0.25 mg/kg. The AUC per dose (AUC_tau) increased linearly from 0.25 to 4 mg/kg (Table 3). Table 4 indicates the time elapsed for plasma cobalt concentration to drop below the regulatory threshold limit in the State of Ohio after administration of various dosages.

Urine cobalt concentrations were highest at 2 hours after dosing for horses receiving 4 and 1 mg/kg IV, whereas C_{max} occurred at 0.25 hours postdosing for the remaining horses. Maximum urine concentrations observed were 73,793; 87,946; 47,826; 36,401; and 24,494 ng/mL in horses receiving 4, 2, 1, 0.5, 0.25 mg/kg, respectively, following the 1st dose. After the 5th dose, maximum urine concentrations were 94,323; 46,466; 38,932; 28,696; and 18,753 ng/mL, respectively, in these same horses. The AUC_{urine} for $CoCl_2$ increased after the 5th dose when compared with the 1st dose, as did urine clearance rate.

Discussion

Intravenous administration of $CoCl_2$ is associated with alterations in variables associated with behavior, cardiovascular physiology and hemodynamics, hematology, urine composition, and endocrine physiology in adult horses, and these effects were observed to be linear by dose. In addition, $CoCl_2$ was shown to accumulate with each dose, which was associated with reduced volume of distribution and reduced plasma clearance. No known benefits are associated with administration of cobalt chloride to healthy horses; in fact, administration of high doses of $CoCl_2$ has been shown here to be potentially harmful to horses acutely. No information is currently available on the effects of chronic administration in this species, although cobalt toxicity has been documented in other species with prolonged exposure (even at low doses, such as with occupational exposures in humans).[1,3]

Cobalt is reportedly given to racehorses in an effort to gain a competitive advantage and enhance their performance on the racetrack; presumably, the bulk of this theoretical benefit lies in the drug's known ability to act as a potent hypoxia mimetic, stabilizing hypoxia-inducible factor 1-alpha (HIF-1α) and enhancing hematopoiesis in other species through increased erythropoietin production. However, there is no published evidence that $CoCl_2$ administration to horses orally or parenterally enhances performance.[7,11] While the hematocrit of the mares included in this study was observed to

increase acutely in response to $CoCl_2$ infusion, values returned to baseline within 15–60 minutes, making this effect on the packed cell volume unlikely to influence performance. As in prior studies,[14] there was no evidence of increased erythropoietin production or a sustained increase in baseline hematocrit over time in response to multiple-dose cobalt administration. Further, the increase in hematocrit noted acutely was not in excess of what has been already clearly established to occur in horses undergoing strenuous exercise[19]; cobalt does not appear to enhance parameters that correlate positively with racing performance.

While little evidence of enhanced hematopoiesis was observed in this study, endocrine and cardiovascular effects that would be associated with risk of harm and adverse effects to the horse and indirectly to human riders and handlers were observed. All mares had increased serum concentrations of cortisol and ACTH shortly after drug administration, suggesting that treatment was associated with robust activation of the hypothalamic-pituitary-adrenal axis and represents a potent physiologic stressor. The mild increase in L-lactate concentrations observed shortly after cobalt administration could have been a response to severe hypertension, tissue hypoxia, or a combination of the two. The increases in cTnI noted within 4–6 hours of drug infusion were in excess of those noted in horses undergoing strenuous physical exercise,[20] similar to values within 12 hours after monensin administration,[21] and might be associated with risk of adverse cardiac events. The arrhythmias that were observed electrocardiographically after each administration in the mare receiving the 4 mg/kg dose (supraventricular tachycardia, paroxysmal ventricular tachycardia, premature ventricular complexes) would also appear to support the presence of this risk. Cobalt is known to induce cardiomyopathy in humans[1] via an unknown mechanism, and the same might be true with chronic administration of cobalt salts to horses; additional longitudinal studies of horses chronically dosed with $CoCl_2$ would be indicated to further investigate this risk. The role that cobalt administration might play in the etiopathophysiology of other arrhythmias that are commonly diagnosed in Standardbred racehorses, such as atrial fibrillation, might also be clarified through larger surveys of racing populations.

An important limitation of the study reported here is the small number of animals subjected to treatment

Table 3. Select pharmacokinetic variables associated with serial intravenous administration of $CoCl_2$ to healthy adult horses at various dosages (4, 2, 1, 0.5, and 0.25 mg/kg).

	Animal ID	Horse 1	Horse 2	Horse 3	Horse 4	Horse 5
Parameter	Dose	4 mg/kg	2 mg/kg	1 mg/kg	0.5 mg/kg	0.25 mg/kg
R^2		0.96	0.92	0.94	0.91	0.90
Lambda_z	1/h	0.003	0.0024	0.0025	0.0022	0.0021
HL_Lambda_z	hr	235	291	275	322	327
C_0	ng/mL	1.12×10^4	5.32×10^3	1.59×10^3	1.29×10^3	3.38×10^2
T_{last}	h	2.23×10^3	2.23×10^3	2.23×10^3	2.23×10^3	2.23×10^3
C_{last}	ng/mL	9.50	13.83	5.90	4.99	2.31
AUCall	h × ng/mL	2.74×10^6	1.26×10^6	4.08×10^5	2.66×10^5	7.95×10^4
AUCINF_obs	h × ng/mL	2.74×10^6	1.27×10^6	4.10×10^5	2.69×10^5	8.06×10^4
AUC_%Extrap_obs	%	0.12	0.46	0.57	0.86	1.35
T_{min}	h	168	168	168	168	168
C_{min}	ng/mL	738	352	79.6	71.0	11.3
C_{avg}	ng/mL	1.45×10^3	6.67×10^2	1.59×10^2	1.44×10^2	24.9
Fluctuation%	%	643	686	849	840	1.18×10^3
CLss	mL/h	8.23×10^3	8.93×10^3	1.88×10^4	1.04×10^4	2.99×10^4
MRTINF_obs	h	1.79×10^3	1.80×10^3	2.48×10^3	1.76×10^3	3.13×10^3
V_z	mL	2.79×10^6	3.75×10^6	7.43×10^6	4.82×10^6	1.41×10^7
V_{ss}_obs	mL	1.47×10^7	1.60×10^7	4.65×10^7	1.82×10^7	9.37×10^7
Accumulation_Index		2.56	3.04	2.89	3.30	3.34
AUC_TAU	h × ng/mL	2.43×10^5	1.12×10^5	2.67×10^4	2.41×10^4	4.18×10^3

R^2, coefficient of determination for the plasma concentrations versus time curve as modeled with nonlinear regression analysis; Lambda_z, terminal rate constant of the plasma concentration versus time curve after 5 IV doses of cobalt chloride to each horse; HL_lambda_z, half-life of the terminal phase of cobalt chloride administered to 5 horses 5 times once weekly; C_0, the extrapolated, zero time plasma concentration of cobalt; T_{last}, last plasma concentration of cobalt measured; C_{last}, last measured concentration of cobalt; AUC, area under the curve including all plasma concentrations; $AUC_{infinity}$, area under the curve extrapolated to infinity by adding the ratio of C_{last}/lambda_z; AUC_%Extrap_obs, the area under the curve, which is added to AUCall and expressed as a percentage of the total AUC (AUC_infinity); T_{min}, time of minimum plasma cobalt concentrations; C_{min}, minimum plasma cobalt concentration; C_{avg}, average plasma concentration of cobalt over 5 dose intervals; Fluctuation%, the fluctuation in plasma concentration from the maximum plasma concentration (peak) to minimum plasma concentration (trough) expressed as a percentage; CL, clearance of cobalt in mL plasma/h over the entire dosing interval; MRTINF_obs, the mean residence time (h) over the entire dosing interval; V_z, volume of distribution associated with the terminal phase; V_{ss}_obs, volume of distribution at steady state; Accumulation_Index, factor relating plasma drug concentrations after a single dose to drug concentrations observed after multiple doses indicated that after 5 doses, peak (trough) concentrations of cobalt would increase in multiples of the accumulation factor; AUC_TAU, area under the curve normalized to the dose interval.

Table 4. Time elapsed for plasma cobalt concentration to drop below regulatory threshold level limit in the State of Ohio (25 ppb; 25 ng/mL) when $CoCl_2$ is administered to healthy Standardbred horses at 5 different doses (4, 2, 1, 0.5, and 0.25 mg/kg). Based on these data, the time to plasma cobalt concentration below regulatory threshold could be as long as 90 days (4 mg/kg dose) or as short as 40 days (0.25 mg/kg dose) after intravenous administration. Values in bold font indicate the time at which plasma cobalt concentration first drops below the regulatory threshold limit for each individual dose.

	$CoCl_2$ Dose (mg/kg)				
	4	2	1	0.5	0.25
Days after last dose	Plasma Co (ppb)				
0	11,183	5,320	1,587	1,289	338
10	5,591.5	2,660	794	644.5	169
20	2,796	1,330	397	323	85
30	1,398	665	198	161	43
40	699	333	99	81	**21**
50	349	166	50	40	11
60	175	83	25	**20**	5.3
70	87	42	**12**	10	2.6
80	44	**21**	6	5	1.3
90	**22**	10	3	2.5	0.66
100	11	5	1.5	1.3	0.33

with each dose (n = 1). While we believe the results to be significant given the magnitude of the hemodynamic responses and the linear dose-response relationship observed after administration, the statistical power of this study does not allow for broad generalizations to be made regarding the physiologic and pharmacologic effects of $CoCl_2$ in larger populations of horses. Additionally, the horses used in this study were not currently in training and were unfit, so the pharmacokinetic and pharmacodynamic effects noted might not be representative of what might occur in athletically trained animals, such as racehorses, when given cobalt salts. The concentration of $CoCl_2$ in the solution prepared by the compounding pharmacy was not analyzed, and therefore, the precise amount of elemental cobalt administered to the horses in this study was not independently verified. Finally, this project did not include evaluation of a control group. Larger studies directed at this target population would be useful for drawing more accurate conclusions about the effects of cobalt salts on athletically trained horses.

In conclusion, intravenous administration of $CoCl_2$ to adult horses is associated with hemodynamic instability and distress as documented by visible discomfort and increase in endocrine markers of severe physiologic stress. Urine discoloration, straining to urinate, and the presence of cellular debris within the urine consisting of epithelial cells and erythrocytes support urinary tract injury, which could have been a direct effect of cobalt or a consequence of severe renal arterial hypertension. While these events appeared to have been transient and reversible, accumulation of cobalt after multiple doses, reduction in plasma cobalt clearance, and reduction in the estimated volumes of distribution suggest changes, which could result in important consequences for animals provided multiple doses long term. These manifestations argue that the effects of $CoCl_2$ are harmful and likely associated with multiple body systems.

Therefore, based on the data presented here, the administration of $CoCl_2$ IV to performance horses represents an animal welfare issue and threatens the well-being of racing animals; administration of $CoCl_2$ at these doses is harmful to horses. These findings are in line with concerns raised by veterinarians and regulatory agencies on the use of cobalt salts in animals used for competitive sport. While additional information on the pharmacodynamic and toxicological effects of this substance in horses would be useful, it appears clear from the information provided that given the lack of currently accepted clinical indication for therapeutic use in this species, cobalt salts should not be administered to horses intravenously at these doses.

Footnotes

[a] 20% $CoCl_2$ injection; Doc Lane's Veterinary Pharmacy, Lexington, KY
[b] Thermodilution balloon catheter 7fr 110 cm AI-07067; Arrow International Inc, Reading, PA
[c] Intramedic PE-240 tubing; Becton Dickinson and Co., Sparks, MD
[d] Intravenous catheter; Terumo Medical Corporation, Somerset, NJ
[e] BD Angiocath, Becton Dickinson Infusion Therapy Systems, Sandy, UT
[f] Foley catheter; BARD Medical, Covington, GA
[g] Mindray Datascope, Mindray North America, Mahwah, NJ
[h] Toshiba Viamo; Toshiba America Medical Systems, Inc., Tustin, CA
[i] Cardiomax III cardiac output computer; Columbus Instruments, Columbus, OH
[j] Optima grade; Thermo Fisher Scientific, Waltham, MA
[k] OmniSolv grade; EMD Millipore Corp., Billerica, MA
[l] Phoenix WinNonlin version 6.4; Pharsight, Cary, NC
[m] ADVIA Centaur cTNI Assay; Siemens Medical Solutions, Inc., Malvern, PA
[n] CUSABIO, College Park, MD
[o] MP Biomedicals, Solon, OH
[p] ACTH Immulite; Siemens Medical Solutions, Inc., Malvern, PA
[q] Roche COBAS c501; Roche Diagnostics, Indianapolis, IN
[r] Stat Profile pHOX Ultra; Nova Biomedical, Waltham, MA

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Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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