Iron is an essential mineral that is critical to many physiological processes including oxygen transport, DNA synthesis, and energy production.1 It is also a critical component for erythropoiesis, particularly in the development of proerythroblasts to mature RBC and hemoglobin synthesis.2 Therefore, overt iron deficiency or functional inability to transport iron will result in anemia.

In health, iron uptake and storage are tightly regulated. When iron stores are inadequate, ferrous iron is transported into duodenal enterocytes via divalent metal transporter 1 and exported into the bloodstream by ferroportin, the only known iron membrane exporter protein.1,3,4 It circulates bound primarily to transferrin and is either immediately used by tissues or stored as ferritin, which most accurately reflects total body iron status.1,3,4 When iron stores are adequate, the protein hepcidin binds to ferroportin, resulting in internalization of the complex, which prevents iron transfer from enterocytes into the blood stream, as well as export from other storage cells.1,3 Hepcidin production is also increased by inflammatory mediators, such as interleukin-6.5 Therefore, in inflammatory disease states, there is limited ability to access intracellular iron stores or absorb iron from the gastrointestinal (GI) tract, resulting in the development of a functional iron deficiency (FID).1,3,6 In both humans and veterinary species, FID is a leading cause of iron deficiency,7–9 resulting in anemia of inflammatory disease.1,3

FID was historically considered relatively refractory to oral iron supplementation due to this hepcidin-induced blockade. Oral iron supplementation is also poorly tolerated due to GI side effects.10–13 Due to reduced GI side effects, ability to bypass the hepcidin blockade, and improved response in human patients

OBJECTIVE
To evaluate IV iron sucrose safety and impact on hematologic and iron indices in healthy cats.

ANIMALS
5 healthy research cats.

PROCEDURES
Cats were administered iron sucrose (0.5 mg/kg, IV) over 30 minutes. Monitoring for acute reactions (temperature, heart rate, respiratory rate, and blood pressure) was performed every 5 minutes during injection and every 15 minutes for an additional hour. Baseline, 24-hour, and 1-, 2-, and 3-week postinjection measurements of CBC with reticulocyte indices, iron panel (ferritin, total iron-binding capacity, and iron), calculated transferrin saturation (TSAT), and serum amyloid A (SAA) concentration were performed.

RESULTS
No cat experienced an acute drug reaction. SAA concentration was increased at 24 hours versus baseline. TSAT and ferritin decreased over time, with 3 cats developing concurrent functional iron deficiency (FID) and anemia. Hct (Spearman correlation \( r_s = 0.805 \)), hemoglobin (\( r_s = 0.770 \)), and reticulocyte hemoglobin content (\( r_s = 0.581 \)) correlated with TSAT.

CLINICAL RELEVANCE
IV iron sucrose was well tolerated in healthy cats but was associated with transient increase in the systemic inflammatory marker SAA. Efficacy evaluation of dose based on iron deficit is needed in sick cats. Despite cumulative blood draw volume below recommended limits, anemia and FID were observed, which has important implications for experimental designs and serial hematologic monitoring. Further evaluation of inflammatory response to IV iron sucrose administration is warranted.
with both absolute and FID, parenteral iron supplementation is recommended. IM-administered iron is absorbed slowly, requiring 1 to 3 weeks for 90% lymphatic uptake. carries similar side-effect risks to IV-administered iron and is less effective in reaching target hemoglobin levels in some human disease processes; therefore, IV supplementation is recommended. High-molecular-weight iron dextran solutions administered IV resulted in serious adverse drug events. However, recent human studies have demonstrated the safety of lower-molecular-weight non-dextran-containing solutions, such as iron sucrose.

FID and anemia have been described in cats with chronic kidney disease and GI disease, and anemia increases all-cause morbidity in sick cats, warranting the need for effective supplementation methods. To the authors’ knowledge, there are no available data on administration of IV iron sucrose in cats. Therefore, the primary objective of this study was to evaluate the safety of IV iron sucrose in healthy cats through serial assessment of clinical parameters and systemic inflammatory markers. A secondary objective was to evaluate the impact of IV iron supplementation on CBC and reticulocyte parameters, as well as serum iron indices.

Materials and Methods

Study protocol

Five castrated male purpose-bred cats (median age, 1.33 years; range, 1.25 to 2.33 years), determined to be healthy by screening physical examination, CBC, and serum biochemistry panel, were enrolled prospectively. Cats were acclimated to their environment for 7 days prior to study initiation (T0). On T0, venipuncture was performed using a 22-gauge butterfly catheter from the medial or lateral saphenous vein for baseline serum amyloid A (SAA) measurement, serum iron panel (iron and ferritin concentrations and total iron-binding capacity [TIBC]), and CBC.

For iron administration, a 20-gauge IV catheter was placed into a cephalic or lateral saphenous vein. Baseline vital parameters (rectal temperature, heart rate, respiratory rate, and indirect blood pressure [Doppler]) were obtained. Cats were administered a 0.5-mg/kg dose of iron sucrose (Venofer; American Regent Inc) IV over 30 minutes. Iron sucrose was diluted to 3 mL total volume in sterile saline (0.9% NaCl) solution, resulting in a final concentration of 0.8 to 0.9 mg/mL administered to all cats. The above listed vital parameters were recorded every 5 minutes during administration and then every 15 minutes for 1 hour following administration. Serum amyloid A measurement, iron panel, and CBC were repeated at 24 hours (T1) and 1 (T2), 2 (T3), and 3 weeks (T4) postinjection. This study was approved by the Institutional Animal Care and Use Committee at Kansas State University (protocol 4441).

Sample collection, storage, and analysis

A maximum volume of 8 mL whole blood was collected from each cat per time point. The maximum blood draw volume was < 5% of circulating blood volume per week based on a circulating blood volume calculation of 55 mL/kg. Blood was immediately divided between EDTA (CBC) and serum (SAA and iron panel) tubes. Blood for serum was allowed to clot at room temperature (20 °C) and then centrifuged (3,500 rpm) for 15 minutes and separated immediately. Serum for SAA was stored at −80°C and analyzed in bulk at study completion using a commercially available immunoturbidometric assay (University of Miami Miller School of Medicine Acute Phase Protein Laboratory). Serum for iron parameters was stored at 4°C and analyzed within 1 wk through the Kansas State University Veterinary Diagnostic Laboratory. A commercially validated feline-specific ELISA (Comparative Hematology Laboratory, Kansas State Veterinary Diagnostic Laboratory) was used to measure ferritin. Transferrin and TIBC were measured using spectrophotometry (Cobas c501 chemistry analyzer; Roche Diagnostics). Transferrin saturation (TSAT) was calculated as iron concentration/TIBC X 100. The CBC with reticulocyte indices were performed immediately through the Kansas State Veterinary Diagnostic Laboratory (ADVIA 2120 Hematology System; Siemens Medical Solutions Inc).

Statistical analysis

Data were assessed for normality using the Shapiro-Wilk test. Due to small sample size and several variables failing normality assumptions, non-parametric tests were used. Data are reported as median (range). The Friedman test was used to compare median vital parameters, hematologic indices, iron indices, and SAA concentration over time, followed by Dunn correction for multiple comparisons, with P < 0.05 considered significant. The Spearman correlation (r) was used to compare iron indices, SAA concentration, and CBC parameters. Strength of correlation was defined as previously described (0 to 0.09 = negligible; 0.1 to 0.39 = weak; 0.4 to 0.69 = moderate; 0.7 to 0.89 = strong; and 0.9 to 1.0 = very strong). Bonferroni corrected P values for multiple comparisons were used.

For statistical analysis, cats were considered anemic if their Hct was below the lower limit of the laboratory reference interval (< 35%). Absolute iron deficiency was defined as a serum ferritin concentration below the lower limit of the reference interval (< 82 ng/mL) and TSAT < 20%. FID was defined as a serum ferritin concentration > 100 ng/mL and TSAT < 20%. Cats with serum ferritin concentrations between 82 and 100 ng/mL and TSAT < 20% were considered to have a combination of absolute and functional iron deficiency. Statistical analysis was performed using commercial software (GraphPad Prism 9.1.2; GraphPad Software).

Results

Clinical tolerance

All 5 cats that were enrolled safely completed the study. No cat experienced significant changes in vital parameters (blood pressure [P = 0.82], heart
rate \( P = 0.28 \), and respiratory rate \( P > 0.99 \); Supplementary Table S1). Median SAA concentration was increased at 24 hours postinjection (3.90 mg/L; range, 1.30 to 27.70 mg/L), compared with that at baseline (1.20 mg/L; range, 0.60 to 8.40 mg/L; \( P = 0.03 \)), but was not associated with adverse clinical findings (Figure 1).

Changes in hematologic indices over time

Values for Hct \( P < 0.01 \), hemoglobin (Hgb; \( P < 0.01 \)), mean cell volume \( P < 0.01 \), and percentage of hypochromic reticulocytes \( P = 0.03 \) decreased during the study period (Figure 2). Over the course of the study, 3 of 5 cats became anemic. In contrast, there was no difference in reticulocyte hemoglobin content (CHR) between baseline and any other study time point \( P = 0.098 \).

Changes in iron parameters over time

Serum ferritin concentrations decreased over time, with lower concentrations at T3 compared with baseline (Figure 3). Values for TSAT insignificantly increased from T0, followed by a decrease over time, with a significant \( P < 0.01 \) difference between T1 and T2. There was no difference in serum iron concentration or TIBC between baseline and any other time point.

Based on the defined ferritin cutoffs for iron deficiency, FID was observed in 4 cats at T2, 2 cats at T3, and 3 cats at T4. No cat developed an absolute iron deficiency based on serum ferritin concentration.

Correlation of hematologic indices and iron parameters

Values for Hct and Hgb were positively correlated with serum iron concentration and TSAT, and CHr was positively correlated with serum iron concentration (Table 1). Although not statistically significant, CHr was generally positively correlated with TSAT and TIBC. Conversely, the percentage of hypochromic reticulocytes was negatively correlated with TIBC. No correlations were observed between serum ferritin concentration and any hematologic parameters. Individual values for hematologic parameters and iron indices for each cat were summarized (Supplementary Table S2).

Discussion

IV iron sucrose administration at a dose of 0.5 mg/kg, administered over 30 minutes following dilution to 0.8 to 0.9 mg/mL, was clinically well tolerated in a small group of healthy cats. Few hematologic changes related to iron sucrose administration were noted. Rather, anemia and a pattern toward iron deficiency developed over 3 weeks. Correlation analysis between hematologic parameters and iron indices reinforced the nonspecificity of routine CBC parameters for iron deficiency.
Significant adverse drug events (AEs) secondary to IV iron administration are rare in humans, with a 0.5% overall incidence of AEs following administration of high-molecular-weight compounds, such as iron dextran. The incidence of life-threatening AEs following administration of these compounds is 0.035%. A metanalysis revealed a lower relative risk (RR) of infusion reactions (compared with a placebo) following administration of lower-molecular-weight formulations (eg, iron sucrose; RR, 1.75; 95% CI, 0.69 to 4.43) versus iron dextran (RR, 3.10; 95% CI, 0.86 to 11.22). Iron sucrose also appears to be well tolerated in humans that have previously experienced AEs following administration of iron dextran.

While uncommon, infusion reactions (eg, flushing, pruritus, or urticaria), neurologic complications (eg, paresthesia, weakness, or back pain), and hypotension have been reported. It should be noted that the risk of some reactions (eg, neurologic AEs) was not increased over placebo following metanalysis, regardless of iron compound. The risk of minor infusion reactions decreases as the stability of carbohydrate complex binding to the iron core increases. Because iron sucrose is considered moderately stable, monitoring for these reactions is still warranted, but none were observed in the present study. More serious AEs (eg, dyspnea or hypotension) are related to both the stability of the iron complex, as well as the speed of infusion. Respiratory rate was an unreliable parameter in this group of cats due to temperament, so subtle changes in tachypnea could have been overlooked. However, because the respiratory rate generally decreased following injection, and respiratory effort remained normal, a respiratory-related AE was considered unlikely. Other AEs that are reported in humans, such as paresthesia or myalgia, may be difficult to detect in feline patients. Progression of AEs has been noted over 24 hours following IV iron sucrose administration in humans. Although vital parameters were recorded for only 1 hour following administration, cats were directly monitored for several hours after infusion and then at the 24-hour time point, with no onset of adverse clinical signs in the intermediate period.

Consistent with what is reported in humans, a transient increase in the inflammatory marker SAA, considered a reliable marker of systemic inflammation in cats, was observed 24 hours after iron sucrose administration. Systemic inflammation secondary to IV iron administration is related to generation of reactive oxygen species. Due to the redox capacity of iron and inclusion in many enzymatic biochemical redox reactions, unbound iron (ie, not bound to transferrin or ferritin) leads to the generation of hydroxyl radicals. Increased markers of systemic inflammation (eg, interleukin-6, hepcidin, or C-reactive protein) have been noted in multiple in vivo and in vitro studies following administration of various IV iron formulations. Many in vitro models utilized much higher doses than are used clinically, which have the potential to overwhelm transferrin-binding capacity, and limit the extrapolation of risk to clinical patients. Importantly, while generation of reactive oxygen species and other inflammatory markers have been noted in human patients, these appear transient. Similarly, in the present study, the increased SAA concentrations were transient and no different at 1, 2, or 3 weeks postinjection of iron sucrose, compared with those at baseline. Additionally, the increased SAA concentrations were not associated with adverse clinical signs (eg, fever).

No significant changes in iron parameters (iron, ferritin, TIBC, or TSAT) were attributed to iron sucrose administration. This finding can be explained, in part, by the study design, as this was not designed as a pharmacokinetic study but rather designed with the objective to determine changes in
hematologic parameters and ferritin concentrations. Iron sucrose is rapidly cleared from the blood, with changes in transient parameters, such as serum iron concentration, TIBC, and TSAT, returning to baseline in < 24 hours. In humans, approximately 70% to 75% of iron sucrose undergoes renal elimination 4 to 24 hours following injection, respectively, with a half-life of 5 to 6 hours. Based on pharmacokinetic calculations, it would be expected that < 0.1 as a fraction of the administered dose would remain in circulation at 24 hours postinjection. Therefore, changes in serum iron, TIBC, or TSAT at any measured time point were not expected. More frequent, short-term sampling would be needed to evaluate pharmacokinetics of IV iron sucrose in cats. Importantly, while there was an insignificant increase in TSAT, neither TSAT nor TIBC (no change) at 24 hours postadministration suggested risk of iron overload at this dose, even in healthy cats without iron deficiency.

Based on human research, it was expected that peak iron sucrose utilization (ie, changes in ferritin) would be noted 2 to 3 weeks following injection and effects on erythropoiesis noted in 3 to 4 weeks. In contrast, both serum ferritin concentration and TSAT decreased, and anemia developed in the majority of these cats over the course of the study. Given the absence of other causes for anemia development, it was most likely iatrogenic due to repeated blood draw, which has important implications for both healthy and sick cats. Although the Institutional Animal Care and Use Committee protocol set the limit for sequential blood draw volume at 5% of circulating volume per week (approx 15 mL per cat), a substantially lower volume (less than or equal to 8 mL) was drawn within that timeframe. The iron deficiency that developed was characterized as functional, based solely on the defined serum ferritin concentrations and a TSAT for FID and absolute deficiency, respectively. Iatrogenic iron deficiency has been reported in humans secondary to repeated phlebotomy, with repeated venipuncture leading to a negative total body iron balance. Over time, as body stores are depleted, the ferritin concentration subsequently decreases and is the most likely cause for the decreasing ferritin and TSAT in this population. While the serum ferritin concentration in the cats of the present study did not decrease below the set threshold (< 82 ng/mL) to be categorized as an absolute deficiency, this might have occurred were repeated venipuncture continued. As SAA concentrations returned to baseline after the 24-hour time point and time to maximum SAA concentration in cats following an inflammatory stimulus is 24 to 48 hours, the effects of serial venipuncture were considered the most likely cause of anemia and FID rather than inflammation secondary to iron sucrose. A control group undergoing only venipuncture would be needed, although iatrogenic anemia has been previously described in cats following repeated blood draws. It should be noted that the study subjects referenced above consisted of sick, hospitalized cats, and comparisons of iron status or inflammatory markers were not performed. Concurrent measurement of additional inflammatory markers (eg, hepcidin) could also further clarify the impact of iron sucrose administration. This consideration is clinically relevant, as an inflammatory response secondary to iron sucrose could negate its beneficial effects.

Consistent with previous literature in dogs and cats, none of the conventional hematologic parameters (eg, mean cell volume) correlated with serum iron parameters. While the percentage of hypochromic reticulocytes has been cited as a more sensitive marker of iron-deficiency anemia in dogs, that variable was not correlated with any conventional CBC parameters (eg, Hct or Hgb), serum iron concentration, or TSAT in this study. This could have been due to low sample size or differences in reticulocyte parameter changes among species, as this value was not different in a previous study between cats with low versus normal serum iron concentrations. In contrast to several previous veterinary studies, CHr did not correlate with any iron parameters in this group of cats. Notably, no conventional CBC parameters or reticulocyte parameters (eg, CHr) correlated with serum ferritin concentration. This highlights the previously demonstrated insensitivity of hematologic parameters to predict total body iron status and underscores the importance of measuring serum ferritin in patients where iron deficiency is a concern. This is particularly important in cats where other methods to distinguish FID from absolute iron deficiency are either not available (eg, hepcidin) or not applicable to this species (eg, bone marrow iron stores).

The present study had several limitations. While IV iron sucrose appeared to be well tolerated in this group of cats, this group was small in number, limiting the ability to screen for rare AEs, which may be observed across a larger population. Furthermore, hypotensive reactions are related to speed of administration and suspected due to exceeding the rate of anaphylotoxin clearance, so safety cannot be extrapolated to shorter infusion times. Secondly, administration of iron sucrose to healthy cats and the dose chosen could have a limited effect on iron indices and hematologic parameters. As there was no iron deficit in this population, the dose was extrapolated from the effective maintenance dose in human pediatric patients. It is possible that a higher per cat dose or dose calculation based on iron deficit in sick cats (total iron deficit = weight [kg] X (target Hb – actual Hb) [g/l] X 2.4 + iron stores [mg]) would have a greater effect. Additionally, human studies suggest that healthy patients have a blunted response to iron supplementation compared with iron-deficient patients.

Assessment of iron sucrose efficacy was confounded by development of anemia and iron deficiency, warranting consideration of sampling protocols in future studies. While the cutoff for anemia was based on laboratory reference range and higher than the definition of anemia in other feline studies, the decreased Hct over the duration of the study remains clinically relevant, even though the magnitude of anemia was not clinically significant.
This study also did not compare IV iron administration to traditional iron supplementation methods in cats (eg, iron dextran). However, our goal was first to evaluate the safety of IV iron, and comparison among iron forms would be an objective for a different study. While IM administration is most common in cats, to the authors’ knowledge, efficacy in sick cats has not been evaluated. Lastly, although sample size likely limited evaluation of correlations between iron parameters and hematologic indices, most findings were consistent with previous literature.

This study provided preliminary evidence that IV iron sucrose is safe in healthy cats at a dose of 0.5 mg/kg when diluted to 0.9 mg/mL and administered over 30 minutes. Future studies screening for AEs in larger groups and evaluating doses based on iron deficit in cats with iron deficiency are warranted. While not the intent of this study, results showed that sequential blood draws below currently accepted limits have the potential to cause iron deficiency and anemia in cats. This finding should be considered both in future research study designs and in hospitalized cats that undergo serial hematologic monitoring over short periods of time.

**Acknowledgements**

Funded by The Kansas State University College of Veterinary Medicine Mentored, Clinical, or Applied Research Grant. The authors thank the Boehringer Ingelheim Veterinary Scholars Program, the Kansas State University Office of Research, and Chet Peterson Family for veterinary student research scholar support (Ullom-Minnich). The authors declare that there were no conflicts of interest.

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