Evaluation of glucose absorption rates following intracoelomic or subcutaneous administration in experimentally dehydrated inland bearded dragons (*Pogona vitticeps*)

Rashea L. Minor

Grayson A. Doss DVM

Christoph Mans Dr Med Vet

From the Department of Surgical Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI 53706.

Address correspondence to Dr. Doss (gdoss@wisc.edu).

OBJECTIVE

To evaluate glucose absorption rates as an indirect measure of fluid absorption after SC or intracoelomic (ICe) administration of 2.5% dextrose solution to experimentally dehydrated inland bearded dragons (*Pogona vitticeps*).

ANIMALS

9 adult bearded dragons.

PROCEDURES

In a randomized, blinded, placebo-controlled, crossover design study, bearded dragons were experimentally dehydrated with a previously described protocol and then received 2.5% dextrose solution (a 1:1 mixture of 5% dextrose in isotonic multiple electrolytes solution; 20 mL/kg), SC or ICe, or a control treatment (the same electrolytes solution without added glucose; 20 mL/kg). Blood glucose (BG) concentrations were measured at predetermined times up to 24 hours after fluid administration. There was a ≥ 14-day washout period between treatments. Mean changes in BG concentration from baseline were compared among treatments.

RESULTS

Administration of 2.5% dextrose solution by either route increased BG concentration with a significantly greater change in values within 5 minutes, compared with control treatment results. The mean change in BG concentration after ICe administration was significantly greater than that after SC administration 15 and 30 minutes after injection, with mean differences of −50 and −36 mg/dL, respectively, for the SC treatment. Within 1 hour after fluid administration, there was no significant difference in BG values between the 2 dextrose administration routes.

CONCLUSIONS AND CLINICAL RELEVANCE

Findings supported that fluid therapy by SC administration, which carries a lower risk of iatrogenic complications, can provide results similar to those achieved with ICe administration.

Dehydration is a common problem in reptiles presented for veterinary care. Fluid therapy is routinely used to replace fluid deficits in reptile patients, and the administration route can vary depending on several factors such as health status and clinician preference. Subcutaneous and intracoelomic (ICe) administration routes are often recommended for fluid therapy of dehydrated reptiles, and the ICe route has been anecdotally reported to provide faster fluid uptake in this situation.[1] However, there is little information available regarding the efficacy of fluid administration by various routes in reptiles, and the ICe route has greater potential for adverse effects, including inadvertent organ, blood vessel, or preovulatory follicle puncture and the restriction of lung expansion with larger fluid administration volumes.[2] Additionally, the efficacy of ICe fluid administration when treating seriously debilitated reptiles, in which coelomic membrane perfusion may be altered or ascites or other coelomic abnormalities may be present,[2] remains unknown. In mammals, intraperitoneal fluid administration results in rapid uptake of large fluid volumes.[3,4] However, this route is rarely used in small animal and human medicine owing to potentially associated hazards and a general lack of research.[5,6]

In previous studies, administration of dextrose-containing (final concentration, 2.5%) fluids produced hyperglycemia after SC administration in inland bearded dragons (*Pagona vitticeps*)[7] and ICe administration in loggerhead sea turtles (*Caretta caretta*).[8] In inland bearded dragons, SC administration of a 2.5% dextrose solution at 50 mL/kg produced marked hyperglycemia that lasted for ≥ 24 hours.[7] Although there is little information available regarding rates of glucose uptake from the coelomic cavity in reptiles, glucose absorption from the peritoneal cavity is rapid in mammals. Intraperitoneal injection of dextrose solution produced an increase in serum glucose concentrations, compared with baseline values, within 10 to 15 minutes in California sea lions (*Zalophus californianus*) and rabbits (*Oryctolagus cuniculus*).[9,10]

The purpose of the study reported here was to compare the rates of fluid uptake after SC and ICe admin-

istration of a 2.5% dextrose solution in experimentally dehydrated inland bearded dragons. We aimed to assess fluid uptake by monitoring changes in blood glucose (BG) concentration as an indicator of fluid absorption. We hypothesized that fluids administered ICe would have more rapid uptake than fluids administered SC.

Materials and Methods

Animals

Nine captive-bred, healthy adult inland bearded dragons (8 males and 1 female; median body weight, 0.3 kg [range, 0.2 to 0.5 kg]) were used in the study. All animals were housed individually in glass aquaria in a climate-controlled room with a 12-hour light-dark cycle. For 12 hours each day, each enclosure received UVB light (ReptiSun 5.0 T5; Zoo Med Laboratories Inc) and the ambient temperature was maintained between 25 and 27 °C. The resulting temperature gradient was a range from room temperature at one end of the tank to approximately 34 °C in the basking area at the other end. The animals were fed gut-loaded insects or mixed leafy greens once daily, 6 d/wk, on a rotating schedule. All animals had ad libitum access to fresh water and were soaked in warm water twice weekly for further water supplementation. All animals were deemed healthy on the basis of results of regular physical examinations, a CBC and plasma biochemical analysis, and daily monitoring. The study was approved by the University of Wisconsin-Madison School of Veterinary Medicine Institutional Animal Care and Use Committee (protocol V005382).

Study design

A randomized, blinded, placebo-controlled, complete crossover design study was performed to compare fluid uptake rates after SC and ICe administration to experimentally dehydrated bearded dragons. All animals were assigned to receive each of the following 3 treatments in a randomized sequence: a 2.5% dextrose solution (a 1:1 mixture of 5% dextrose in isotonic multiple electrolytes solution [Plasma-Lyte A]) that was previously found to result in hyperglycemia after SC administration to bearded dragons,[7] administered ICe; the same 2.5% dextrose solution, administered SC; and the same isotonic electrolytes solution (Plasma-Lyte A) without added glucose, administered ICe as a negative control for the BG measurements. Randomization was performed with online software (Research Randomizer version 4.0; Geoffrey C. Urbaniak and Scott Plous). A single dose of the assigned fluid (20 mL/kg, calculated on the basis of each animal's body weight prior to experimentally induced dehydration) was administered as a bolus in each experiment. Both the individual performing the ICe injections and the individual performing venipuncture were blinded to the ICe solution administered. There was a minimum washout period of 2 weeks between experiments.

Beginning 48 hours prior to fluid administration, furosemide (Salix; 10 mg/kg, SC, q 12 h for 4 doses) was administered to each bearded dragon in the axillary area to induce dehydration according to a previously published protocol.[11] Water dishes were removed from the enclosures at the time of the first furosemide injection. Food was withheld for 36 hours before fluid administration in each experiment, and no greens were fed in the meal before the withholding period began. During each experiment, no food, water, or soaking was provided until 24 hours after the described fluid bolus administration.

Experiments were performed in 1 room with the ambient temperature maintained between 25 and 27 °C. All animals were sedated with alfaxalone (Alfaxan Multidose; 15 mg/kg, SC), administered in the axillary area, to facilitate fluid administration and avoid iatrogenic injury during ICe injection. Then, a blood sample (0.05 to 0.1 mL) was collected from the caudal vein of the sedated animal with an insulin syringe attached to a 29-gauge needle for baseline BG measurement. Following blood sample collection, the SC and ICe fluid administration sites were aseptically prepared, and the same-size needle (25 gauge) was used for both injection routes. Subcutaneous injections were performed in the laterodorsal region of the body wall, immediately caudal to a forelimb and contralateral to the alfaxalone injection site. Intracoelomic injections were performed under guidance of an ultrasound machine equipped with a linear transducer probe (L8-18i-SC; GE Healthcare) to prevent inadvertent injection into a vessel, coelomic organ, or coelomic fat pad. Intracoelomic fluid injections were performed in the left caudal aspect of the coelom, immediately lateral to midline with the animal held in dorsal recumbency. After needle insertion, the syringe was aspirated for evidence of blood or air. When negative pressure was obtained, the full fluid volume was administered over < 10 seconds. Additional blood samples (0.05 to 0.1 mL each) were collected 5, 15, 30, 60, and 240 minutes and 24 hours after fluid bolus administration. All BG measurements, including those at baseline, were performed on the same blood sample in duplicate with 2 point-of-care glucometers (Accu-Chek Aviva; Roche) of the same model. All measurements were performed on whole blood from the syringe. Duplicate measurements were performed < 60 seconds apart, and the mean value was used for analysis. If the 2 measurements varied by > 20%, a third measurement was performed, and the mean of the 3 values was used. Glucometers were calibrated in accordance with the manufacturer's guidelines. Immediately after the final BG measurement, bearded dragons were soaked in warm water, and food and drinking water were provided. All animals were monitored for signs of adverse effects throughout and after the procedures.

Statistical analysis

Data were analyzed with commercial statistical analysis software (SigmaPlot; Access Softek). The data were tested for normality with the Shapiro-Wilk test and for equal variance with the Brown-Forsythe test, and test assumptions were met. The mean changes

in BG concentrations from baseline were compared among treatments by means of 2-way repeated-measures ANOVA. The Holm-Sidak method was used for post hoc pairwise comparisons when significant differences were determined by ANOVA. Values of $P < 0.05$ were considered significant.

Results

Mean ± SD baseline BG concentrations were 143 ± 36 mg/dL, 153 ± 17 mg/dL, and 146 ± 35 mg/dL prior to administration of 2.5% dextrose SC, 2.5% dextrose ICe, and the control treatment (ICe), respectively. A third BG measurement was performed for 18 of 189 samples collected (the total number of samples for 7 time points/animal/treatment, including baseline). Administration of the control treatment did not result in a significant ($P > 0.94$) change in mean BG concentration from the baseline value at any time point. Both SC and ICe administration of 2.5% dextrose solution resulted in increased BG concentrations, with a significantly greater mean change from baseline ≤ 5 minutes after injection, compared with results for the control treatment **(Figure 1)**. The ICe treatment resulted in significantly greater mean increases in BG concentration 15 and 30 minutes after injection, compared with that after SC administration. The mean difference in this change after SC administration, compared with ICe administration, was −50 mg/dL (95% CI, −91 to −9 mg/dL; $P < 0.001$) and −36 mg/dL (95% CI, −73 to 0 mg/dL; $P = 0.004$) 15 and 30 minutes after bolus administration of the fluid, respectively. Within 1 hour after fluid administration, there was no significant difference in the mean change from baseline between treatments administered by the 2 routes.

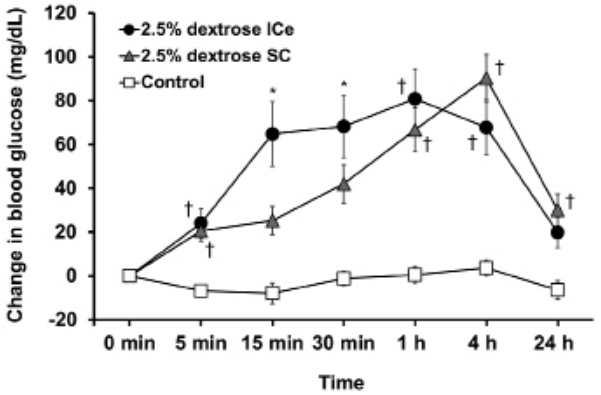

Figure 1—Mean ± SEM changes in blood glucose concentrations in 9 experimentally dehydrated adult inland bearded dragons (*Pogona vitticeps*) following administration of a 2.5% dextrose solution (a 1:1 solution of 5% dextrose in multiple electrolytes solution) SC, the same 2.5% dextrose solution intracoelomically (ICe), or a control treatment (the same multiple electrolytes solution without added glucose) ICe in a randomized crossover design study with a ≥ 2-week washout period between treatments. The dose for all treatments (20 mL/kg) was administered as a bolus injection. The baseline value was obtained immediately prior to each treatment (recorded as 0 minutes). *Within a time point, the changes in blood glucose concentration differ significantly ($P < 0.05$) among all treatments. †Within a time point, the changes in blood glucose concentration differ significantly ($P < 0.05$) from control group.

Discussion

The SC route has been historically discouraged for fluid administration in dehydrated reptiles, with other routes such as ICe, IV, and intraosseous recommended over concerns of poor vascularity of the SC space, compared with that in mammals.[12,13] In contrast, the ICe route has been suggested to result in faster fluid uptake than the SC route in reptiles.[1,14] In the present study, both SC and ICe routes of administration for 2.5% dextrose solution resulted in increased BG concentrations in experimentally dehydrated inland bearded dragons within 5 minutes, indicating that with both routes the onset of glucose absorption was rapid. Although there is little published information on rate of glucose or fluid absorption following SC or ICe fluid administration in reptiles or birds, the rate of increase in BG concentration following both SC and ICe administration of 2.5% dextrose solution to the bearded dragons in this study was comparable to results in mammals. In mice, SC administration of 5% dextrose solution resulted in a significant increase in BG concentration ≤ 5 minutes after injection.[15] Intraperitoneal administration of 5% dextrose solution in rabbits resulted in a significant increase in BG concentration within 10 minutes,[10] and intraperitoneal injection of 20% dextrose solution in sea lions produced a significant increase in BG concentration within 15 minutes.[9] The rapid increase in BG concentration following SC injection of 2.5% dextrose solution in the present study contested claims that the SC route of administration is unimportant in reptile medicine.

Differences in the observed mean changes in BG concentrations over time suggested a distinction in glucose absorption kinetics between administration routes. Administration of 2.5% dextrose solution SC resulted in a less rapid increase to maximum BG values, compared with that after ICe administration, for which BG values generally plateaued 15 minutes after administration. These discrepancies could have been attributable to less robust vascularization of the SC space and subsequent delayed absorption, compared with that from the coelomic cavity. Additionally, fluids administered into the coelom are exposed to a much larger absorptive surface area than those deposited in SC sites. In mammals, the peritoneal membrane is an extremely large absorptive surface area, with both parietal and visceral peritoneum contributing to fluid and solute exchange.[16] The faster rise to maximum BG values after ICe administration provided valuable insight into the differences in absorptive capacity between the 2 routes.

The present study had several limitations. First, although used as a marker of fluid absorption rates in these experiments, the absorption of glucose and subsequent changes in BG concentrations did not provide a measurement of the fluid absorption rates from a volume perspective. Transport of fluids and solutes across the peritoneal membrane is a complex process, governed by numerous factors, including characteristics of the administered fluid; surface area; oncotic, osmotic, and hydrostatic pressures; membrane permeability; and other patient-specific factors.[17] Evaluation of free water

movement following fluid administration in vivo is logistically challenging, and to our knowledge, a model to assess this variable in reptiles has not been developed. Additionally, the differences in reptilian physiology, particularly fluid homeostasis, make direct comparisons with mammalian research difficult. However, because water is freely permeable across all membranes, including the vascular endothelium,[18,19] it was likely that some degree of water absorption had already occurred with both administration routes by the time that a significant increase in BG concentration was detected (5 minutes after injection). Additional studies investigating the rates of fluid uptake after administration to reptiles by various routes would be beneficial. Another consideration is that the bearded dragons in this study were experimentally rather than spontaneously dehydrated, which may have influenced the results. The rates of glucose and water uptake following fluid administration may differ in severely dehydrated animals or in animals in hypovolemic shock or with underlying coelomic abnormalities. Additionally, bearded dragons are well adapted to arid conditions and may therefore use exogenous fluids differently than reptile species that are normally found in and adapted to other climates. Studies investigating differences in fluid use among species with disparate environmental niches are needed. Finally, there are presently no point-of-care glucometers that have been validated for use in any reptile species. Interpreting a single point-of-care glucometer BG reading in a sample from a reptile is ill-advised; however, the study reported here evaluated changes in BG concentrations over time, and we assessed each sample from each time point in duplicate with 2 separate machines. The same 2.5% dextrose solution used in the present study was previously shown to increase BG concentrations over time when measured with a benchtop analyzer in bearded dragons following the same experimental dehydration protocol,[7] supporting that the increases in BG detected by the glucometers likely reflected true increases in BG concentrations. Also, the lack of significant changes in BG concentrations following administration of the control treatment (which did not contain glucose) in both the present study and the aforementioned investigation[7] supported this.

The findings of the study reported here may be relevant when treating hypoglycemic reptiles. However, considering that the bearded dragons in this study were experimentally dehydrated and not hypoglycemic, caution should be used when extrapolating the results to clinical practice. Regardless of the administration route, 2.5% dextrose solution administration induced a persistent increase in BG concentrations in these animals for ≥ 24 hours, although the mean values remained within the range of expected values reported for the species.[20] This result was similar to findings for bearded dragons and loggerhead sea turtles, in which administration of dextrose-containing fluids resulted in long-lasting increases in BG concentrations.[7,8]

In experimentally dehydrated bearded dragons, SC and ICe administration of 2.5% dextrose solution resulted in rapid and persistent increases in BG concentrations. Although the initial increases in BG values were similar between routes, the increase over time was slower for the SC route, with a more rapid rise and plateau of BG values following ICe administration. Future studies investigating effects of administration route and fluid composition on fluid absorption in naturally dehydrated reptiles would be beneficial.

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