Technical Note: Using calcium carbonate as an osmolar control treatment for acid-base studies in horses

L. P. Frey*, K. H. Kline*1, J. H. Foreman†, and J. T. Lyman*

Departments of *Animal Sciences and †Veterinary Clinical Medicine, University of Illinois, Urbana 61801

ABSTRACT: The efficacy of using calcium carbonate as an osmolar control treatment for acid-base studies in horses receiving alkalinizing compounds was evaluated. Six mares were nasogastrically intubated with isomolar quantities of sodium or calcium as sodium bicarbonate or calcium carbonate or with water during three treatment periods. Doses of the carbonic acid salts were 500 mg/kg sodium bicarbonate mixed with 4 L of distilled water (positive control) and 595 mg/kg calcium carbonate mixed with 2 L of distilled water to yield isosmolar treatments. Four liters of distilled water served as the negative control. Jugular venous blood samples were drawn before intubation and at hourly intervals for 6 h after intubation. The serum electrolytes Na+ and K+, blood pH, and HCO3− were determined. The sodium bicarbonate treatment increased blood pH and HCO3− (P < 0.01) above both the water and CaCO3 treatments. No differences (P > 0.05) were found between the water and CaCO3 treatments. These data indicate that calcium carbonate may serve as a suitable osmolar control treatment for studying the effects of treatments that affect acid-base status of horses.

Key Words: Acid Base Disorders, Alkalinization, Horses, Osmolarity


Introduction

Many studies have been conducted in horses examining the effects of induced acid-base changes. Within the past decade, metabolic alkalosis resulting from sodium bicarbonate administration has been studied extensively, due to the potential for induced alkalosis to affect racing performance. Using a negative control in these studies is pertinent but not always practiced. Water is the most common solvent of most salts and is typically used in dissolving sodium bicarbonate prior to nasogastric intubation, but water alone cannot serve as an osmolar control. Although it serves as a useful isovolemic control, the difference in osmolarity between a water control and an experimental sodium bicarbonate treatment may affect the rate of absorption of each treatment.

The purpose of this study was to compare calcium carbonate (CaCO3) and water as controls for evaluating the effects of an administered alkalinizing compound. Calcium carbonate is a carbonic acid calcium salt that is odorless and tasteless. Commercial CaCO3 is produced by chemical means and is 98 to 99% pure and can be purchased in powder or crystalline form. This product has been consumed by humans as an antacid, for supplementing calcium, and in veterinary medicine as an antidiarrheal agent. In this study, it was hypothesized that CaCO3 would not significantly change the blood acid-base balance in horses. This hypothesis was tested by administration of CaCO3, water, and sodium bicarbonate to six horses.

Materials and Methods

Animals

Six mares (one Thoroughbred, three Standardbreds, and two Quarter Horses) were studied for three periods over 2 wk in a 3 × 3 Latin square design with two replicates. The mares (477 to 630 kg) were housed in outdoor paddocks or pastures when not being studied and were allowed to consume mixed alfalfa-grass hay and water on an ad libitum basis. On study days, mares were placed in individual stalls and not allowed feed or water during the treatment and sampling period. The protocol was approved by the University of Illinois Animal Care and Use Committee.

Treatments

Doses of treatments were 500 mg/kg of sodium bicarbonate (positive control) or 595 mg/kg of calcium carbonate, yielding isomolar doses of 5.95 mmol sodium or calcium/kg BW. The sodium bicarbonate treatment
was mixed with 4 L of distilled water and the calcium carbonate treatment was mixed with 2 L of water to yield iso-osmolar solutions. Four liters of distilled water was used as the water treatment (negative control). There were three periods in this study, each separated by 1 wk. In each period, two horses were administered the same treatment. Treatments were assigned randomly to treatment letters and switched according to preplanned Latin square treatment.

**Sampling**

Venous blood samples were taken by jugular venipuncture prior to intubation and at 1-h intervals for 6 h. Samples for blood gas analysis were collected anaerobically into evacuated lithium heparinized vacutainers, placed on ice, and analyzed within 1 h for pH and HCO$_3^-$ concentration on an automated blood gas analyzer (CIBA-Corning 238 blood gas analyzer, Hitachi, Ltd., Tokyo, Japan). The blood gas analyzer was calibrated manually once daily and automatically hourly.

Samples for serum electrolyte determinations were collected in evacuated plain glass tubes and allowed to clot at room temperature before centrifugation and serum separation. Serum electrolyte concentrations of Na$^+$ and Ca$^{++}$ were determined by ion-specific electrodes in a serum chemistry analyzer (Hitachi 911 automatic analyzer) that was calibrated manually once daily and automatically hourly.

**Statistical Analysis**

Analysis of variance for repeated measures was employed using the General Linear Models procedure of SAS (SAS Inst. Inc., Cary, NC). Sources of variation accounted for were among treatments and horses within treatments, and the latter was used as the error term. Mean comparisons among treatments were made using the F-test for significance of differences among treatments within time periods.

**Results**

Results indicated that ingestion of sodium bicarbonate increased blood bicarbonate and blood pH ($P < 0.01$) above the water and CaCO$_3$ treatment (Figures 1 and 2). Sodium bicarbonate resulted in a nonsignificant increase in blood sodium ($P = 0.19$) (Figure 3) and decrease in blood calcium ($P = 0.08$) (Figure 4). No differences ($P > 0.05$) were detected in blood bicarbonate or pH between the water and CaCO$_3$ treatments (Figures 1 and 2). Serum sodium and calcium values did not differ significantly ($P > 0.05$) between the water and calcium carbonate treatments (Figures 3 and 4).

**Discussion**

Experimental protocols testing the effects of a drug treatment on blood constituents are usually designed
with a control group. In this experiment, we examined a negative control water treatment, a calcium carbonate treatment, and a positive control treatment of sodium bicarbonate. Our objectives were to administer isomolar and iso-osmolar concentrations of sodium bicarbonate and calcium carbonate to horses and make comparisons to a conventional water control treatment given to those same horses.

Many studies in the past have examined the effects of oral sodium bicarbonate in horses using water as a control (Greenhaff et al., 1990; Hanson et al., 1993; Frey et al., 1995). In these studies, sodium bicarbonate was dissolved in 2 to 4 L of water prior to nasogastric intubation. The authors used an equal volume of water in both the control and sodium bicarbonate treatment to eliminate treatment volume as a confounding variable when examining the effects of sodium bicarbonate. Unlike in human studies, a placebo treatment such as sodium chloride or calcium carbonate was not necessary to mask the treatments to eliminate any psychological effects on performance. For the objectives of these previous experiments, water served as an acceptable control for determining the effects of sodium bicarbonate on acid-base balance and performance.

Calcium carbonate has been administered as an oral placebo in human exercise studies (Wilkes et al., 1983; McNaughton, 1990; McNaughton and Cedaro, 1992). In addition to the placebo and experimental treatment, these studies also examined absence of treatment as a control. The dose of calcium carbonate for these studies was 300 to 400 mg/kg BW. These studies were designed to examine the effects of sodium bicarbonate or sodium citrate taken orally on acid-base status and exercise performance of various durations and intensities. Their results for the placebo and control groups did not differ. It was reported that no acid-base differences were found between the control and placebo during the post-ingestion measurement period, suggesting that the placebo had no effect. These results are in agreement with the current study in which a higher dose of calcium carbonate was used.

Sodium chloride, ingested in small doses, has also served as a placebo control in several human studies (Katz et al., 1984; Kowalchnk et al., 1989; Hausswirth et al., 1995). The doses of sodium chloride used in these studies ranged from 45 mg to 1.0 g/kg BW. These studies also investigated the effects of induced alkalosis on acid-base status and exercise performance. It was reported that the doses used did not influence acid-base status and were successful in masking the identities of the treatments. One study in horses (Hinchcliff et al., 1993), however, used a larger dose of sodium chloride (0.7 g/kg BW) on an equimolar basis to sodium bicarbonate (1 g/kg BW). It was reported that the sodium chloride decreased arterial blood pH and horses became acidotic. This suggests that sodium chloride does not

**Figure 2** Venous plasma pH after oral administration of sodium bicarbonate, calcium carbonate, and water. Values represented are means ± SE.
Figure 3. Venous plasma sodium after oral administration of sodium bicarbonate, calcium carbonate, and water. Values represented are means ± SE.

Figure 4. Venous plasma calcium after oral administration of sodium bicarbonate, calcium carbonate, and water. Values represented are means ± SE.
serve as an appropriate equimolar control when determining the effects of alkalizing compound on acid-base balance in horses.

Implications

Calcium carbonate may serve as a more appropriate control than water when studying and subsequently interpreting acid-base alterations from oral administration of alkalizing compounds. Results of this study indicate that calcium carbonate, given orally, does not significantly perturb the acid-base status of horses and can be given in isomolar concentrations in comparison to experimental treatments. Additionally, treatment solutions can be made to be iso-osmolar. This would allow for direct comparison of the anion effects of various sodium salts administered by standardizing the concentrations of strong ions between treatments. Using calcium carbonate as a control in future studies of horses during rest and exercise may help clarify the mechanisms of alkalizing compounds on acid-base balance.

Literature Cited


