Intracellular magnesium concentrations in dogs with gastric dilatation-volvulus

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Objective—To quantify and compare intracellular magnesium concentrations (Mg_i) in clinically normal dogs (control dogs) and dogs that have gastric dilatation-volvulus (GDV dogs) and to determine whether there is a difference in Mg_i and serum magnesium concentrations (Mg_s) between GDV dogs with and without cardiac arrhythmias.

Animals—41 control dogs and 21 GDV dogs.

Procedure—Rectus abdominis muscle specimens were obtained from control and GDV dogs for determination of Mg_i. Blood samples were obtained from GDV dogs for determination of Mg_s, and dogs were monitored for 48 hours for cardiac arrhythmias. Muscle specimens were frozen at –40 C, oven dried at 95 C, and digested with concentrated nitric acid. Multielemental analyses were performed by simultaneous/sequential inductively coupled plasma-atomic emission spectroscopy with fixed-cross flow nebulization. The Mg_i was standardized to sulfur content to correct for the amount of fat and fascia in the muscle specimen. Mean (± SEM) values were recorded in parts per million (ppm).

Results—There were no significant differences in Mg_i between control (627 ± 11.1 ppm) and GDV (597 ± 20.5 ppm) dogs, in Mg_i between GDV dogs with (590 ± 34 ppm) and without (584 ± 29 ppm) cardiac arrhythmias, and in Mg_s between GDV dogs with (1.77 ± 0.26 ppm) and without (1.51 ± 0.09 ppm) cardiac arrhythmias. There was no correlation between Mg_s and Mg_i (R^2=0.0001).

Conclusions and Clinical Relevance—Results indicate that Mg depletion is not pathophysiologically important in dogs with GDV and does not play a role in the cardiac arrhythmias detected in these patients. (*Am J Vet Res* 2000;61:1415–1417)

Gastric dilatation-volvulus (GDV) is an acute disease of large breed dogs that is associated with a mortality of 18 to 33%.[1,2] There are numerous theories regarding the cause of GDV, and many physical and environmental factors have been reported to increase the risk of GDV. Some dogs with GDV develop myocardial ischemia and necrosis,[3] and 60 to 70% of dogs with GDV develop cardiac arrhythmias.[3,4] Despite many years of research, the exact cause of these complications is not known; it has been postulated that electrolyte abnormalities, reductions in cardiac output, cardiac contractility, and coronary arterial perfusion play key roles.[4]

Recent observations in human critical care facilities have demonstrated alterations of **magnesium (Mg)** balance in critically ill patients.[5-8] In certain studies, up to 50% of intensive care unit patients had low **intracellular magnesium concentrations (Mg_i)**.[5,7] Low Mg concentrations are observed with a number of different endocrine, gastrointestinal, and renal disease processes as well as certain types of drug therapy and chronic alcoholism. Abnormalities and complications associated with low Mg_i include increased mortality, prolonged stay in the intensive care unit, cardiac arrhythmias, myocardial necrosis, and muscle weakness.[5-7,9,10]

Complications such as cardiac arrhythmias and myocardial necrosis are observed in dogs with GDV. Low Mg_i may develop in dogs with GDV and may be associated with these cardiac changes. Magnesium is the second most abundant intracellular cation.[6] Only approximately 1% of the total body Mg is in the serum with approximately 20% in skeletal muscle, 60% in bone, and the remainder in other tissues, primarily the heart and liver. Assays for **serum magnesium concentrations (Mg_s)** are, thus, insensitive predictors of total body Mg status. In fact, studies in people have concluded that Mg_s within reference range does not preclude total body Mg depletion, and there is no significant correlation between Mg_s and Mg_i.[7]

To our knowledge, there have been no measurements of Mg_i in clinically normal dogs, dogs with GDV, or dogs with other diseases. The objective of this study was to quantify and compare Mg_i in clinically normal dogs and dogs with GDV. A secondary objective was to determine whether there was a relationship between Mg_i concentrations and cardiac arrhythmias in dogs with GDV. Our hypothesis was the null hypothesis. It was hypothesized that there would be no difference between Mg_i and Mg_s measured in muscle specimens and blood samples taken from clinically normal dogs and dogs afflicted with GDV. Our secondary hypotheses were that there would be no relation between cardiac arrhythmias and Mg_i or Mg_s, and there would be no correlation between Mg_i and Mg_s in clinically normal dogs and dogs afflicted with GDV.

Materials and Methods

The All University Committee on Animal Use of Michigan State University approved this study. Clinically normal (control) dogs in our study were used in the student surgery laboratory for nonrecovery abdominal surgery. These 41 control dogs were premedicated with acepromazine (0.1

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to 0.2 mg/kg of body weight, IM) and atropine (0.02 mg/kg, IM). Anesthesia was induced with thiopental (6 mg/kg up to 20 mg/kg total dose, IV) and maintained on isoflurane in oxygen inhalant anesthesia. Using a standard ventral midline laparotomy, a specimen of the rectus abdominis muscle was obtained from each dog. These specimens weighed approximately 1 g. All muscle specimens were frozen at –40 C for later analysis of Mg_i.

Dogs admitted to the Michigan State University Veterinary Teaching Hospital Emergency Service from Jun 1997 to Jul 1998 because of GDV (GDV dogs) were included in our study. A diagnosis of GDV had been made on the basis of history, physical examination findings, and results of abdominal radiography. All GDV dogs, at the time of admission, received standard initial treatment that consisted of aggressive administration of fluids intravenously and attempts at gastric decompression, using an orogastric tube or percutaneous trocharization. Venipuncture was performed, and blood was collected in a heparinized syringe, Ca EDTA tubes, and serum separator tubes and submitted for blood gas analysis, routine hematologic evaluation, and serum biochemical analysis, respectively. In GDV dogs for which the owners elected euthanatization, a rectus abdominis muscle specimen was obtained immediately following a lethal dose of sodium pentobarbital, IV.

For all other GDV dogs, anesthesia protocol was at the discretion of the anesthesiologist. At the time of surgery for correction of GDV, a 1-g muscle specimen was obtained from the rectus abdominis muscle via ventral midline laparotomy. All muscle specimens were frozen at –40 C for later analysis of Mg_i. Dogs were monitored in the postoperative period for cardiac arrhythmias by obtaining a continuous ECG for 48 hours. If the dog was intractable and continuous ECG monitoring was impossible, the ECG was monitored on an hourly basis for 10-minute periods throughout the 48-hour postoperative period. All cardiac arrhythmias were recorded and described.

Muscle specimens from 41 control dogs and 21 GDV dogs were frozen at –40 C, then batch sampled for tissue mineral analysis. Muscle specimens were trimmed of excess fat and connective tissue, placed in a polytetrafluoroethylene container, and oven dried for 5 hours at 95 C. The specimens were weighed on an analytical balance by transferring to a tared weigh paper and placed back into the original container. Two milliliters of concentrated nitric acid were then added to the container, and the specimens were digested overnight at 95 C. The digest was quantitatively transferred with water to a 10-ml volumetric flask and brought to volume. Multielemental analyses were performed by simultaneous/sequential inductively coupled plasma-atomic emission spectroscopy with fixed-cross flow nebulization, following accepted procedures.[11,12] The Mg_i was standardized to sulfur content to correct for the variable amount of fat and fascia in the muscle specimens. Results are presented as mean (± SEM) in **parts per million (ppm)**. The detection limit of the assay was 0.3 ppm, and the coefficient of variance was < 1%.

While testing for Mg content by tissue mineral analysis, we also obtained measurements of **calcium (Ca)**, **copper (Cu)**, **iron (Fe)**, **phosphorus (P)**, **zinc (Zn)**, **sodium (Na)**, **potassium (K)**, **and sulfur (S)**. These values were also standardized to sulfur content.

Statistical analysis—The Mg_i between control and GDV dogs was compared by a Student *t*-test. The Mg_i and Mg_s between GDV dogs with and without cardiac arrhythmias were compared, using a Student *t*-test. The correlation between Mg_i and Mg_s was measured. The correlation between arterial P_{CO_2}, P_{O_2}, and pH values and Mg_i and Mg_s were also measured. Values for arterial P_{CO_2}, P_{O_2}, and pH were compared between GDV dogs with and without cardiac arrhythmias by a Student *t*-test. Values for Ca, Cu, Fe, P, Zn, Na, and K concentrations were compared between control and GDV dogs and between GDV dogs with and without cardiac arrhythmias by a Student *t*-test. Statistical significance was defined as a P value < 0.05. The **degree of correlation (R^2)** was reported.

Results

Of the 21 GDV dogs, 2 were euthanatized at the time of admission, and 1 was euthanatized during surgery because of severe gastric and splenic necrosis. The remaining 18 GDV dogs survived surgery and the postoperative period and were discharged from the hospital. Mean Mg_i in the 41 control dogs was 627 ± 11.1 ppm. Mean Mg_i in the 21 GDV dogs was 597 ± 20.5 ppm. This difference was not significant (P = 0.22).

Seven of the 18 surviving GDV dogs developed cardiac arrhythmias; all were ventricular arrhythmias. The 1 GDV dog that was euthanatized during surgery also had a ventricular arrhythmia. One GDV dog had ventricular tachycardia, and 7 had intermittent ventricular premature contractions. Mean Mg_i for GDV dogs with cardiac arrhythmias was 590 ± 34 ppm, and mean Mg_i for GDV dogs without cardiac arrhythmias was 584 ± 29 ppm (P = 0.89). The Mg_s for GDV dogs with and without cardiac arrhythmias was 1.77 ± 0.26 mEq/L and 1.51 ± 0.09 mEq/L, respectively. The difference was not significant (P = 0.38).

The Mg_s was measured in 18 GDV dogs. Mean Mg_s of GDV dogs was 1.61 ± 0.13 mEq/L, which is within the hospital reference range (1.24 to 1.91 mEq/L). There was < 1% correlation between Mg_s and Mg_i (R^2 = 0.0001).

There were no significant differences in tissue Ca, Cu, Fe, P, Zn, Na, K, and S contents between the GDV dogs and control dogs or between GDV dogs with and without cardiac arrhythmias. An arterial blood gas analysis was performed in 13 GDV dogs. There was no correlation between Mg_i or Mg_s and arterial P_{O_2}, P_{CO_2}, or pH (all $R^2 < 0.16$). There were no significant differences in arterial P_{O_2}, P_{CO_2}, or pH between GDV dogs with and without cardiac arrhythmias.

Discussion

In human critical care units, hypomagnesemia has been observed in 20 to 65% of patients. This high occurrence of hypomagnesemia in people may be the result of chronic alcoholism, uncontrolled diabetes mellitus, loss of renal or gastrointestinal tract function, acute pancreatitis, aggressive fluid therapy, use of diuretics and cardiac glycosides, and, rarely, inadequate dietary intake of Mg.[5,6,8] It has also been demonstrated that the mortality rate of hypomagnesemic patients is twice that of those patients with Mg concentrations within the reference range.[5] In people, the effects of Mg depletion are most often manifested by cardiovascular abnormalities. Some of the cardiovascular abnormalities found in patients with Mg depletion include premature atrial and ventricular systoles, sustained atrial fibrillation, and ventricular tachycardia.[5] These arrhythmias can occur in patients with no known history of cardiac disease, are resistant to traditional antiarrhythmic therapy, but are terminated by parenteral infusions of Mg. Magnesium also appears to have a role in ischemic heart disease. Results of Mg retention tests indicate that there is a much higher retention in patients with ischemic heart disease than

in clinically normal patients.[5] In an in vitro study, isolated coronary arteries from dogs had a greater spasticity in media with low Mg concentrations, compared with those with physiologic Mg concentrations.[13]

Because low Mg concentrations have been associated with increased mortality, cardiac arrhythmias, and muscle weakness, it was attractive to speculate that low Mg concentrations may play a role in the pathophysiologic changes found with GDV, a disease associated with increased mortality, cardiac arrhythmias, and (theoretically) stomach muscle weakness. We found that there were no differences in Mg_i of GDV versus clinically normal dogs, that Mg_s in GDV dogs were within the reference range, and that there were no differences in the Mg_i or Mg_s between GDV dogs with and without cardiac arrhythmias.

Only 3 of 21 GDV dogs died during our study, and all 3 were euthanatized per owner request. For this reason, it was not useful to attempt to correlate Mg concentrations with mortality rate in GDV dogs. The mortality rate for GDV dogs in our study (13.6%) was lower than that previously reported for dogs with GDV (18 to 33%).[1,2]

Results of previous studies indicate that Mg_s does not correlate with Mg_i.[7] We did not find a significant correlation between Mg_s and skeletal muscle Mg_i in the GDV dogs.

In our study, we used skeletal muscle to determine Mg_i. Skeletal muscle provides an excellent and accepted measure of Mg_i.[7] However, it is conceivable that skeletal muscle Mg_i may differ from cardiac muscle Mg_i and, more importantly, that skeletal versus cardiac muscle may respond differently to disease. However, we are unaware of data comparing Mg_i in canine skeletal and cardiac muscles. In the rat subjected to diuretic therapy as a means to decrease Mg concentrations, the reductions in skeletal and heart muscle Mg_i were similar.[14]

Whereas skeletal muscle biopsy is an accepted and accurate technique for obtaining tissue specimens for the determination of Mg_i, it is usually an inefficient means of making a diagnosis of Mg depletion. It requires general anesthesia, or heavy sedation and local anesthesia, a small surgical approach, and a delay in processing, diagnosis, and initiation of therapy. In our study, muscle biopsy was easy, efficient, and free of complications as the dogs were already under general anesthesia and undergoing an abdominal surgical procedure.

A method has been developed for determination of Mg_i from blood mononuclear cells[15]; however, the results of these determinations have not been found to correlate well with Mg_i in body tissues of critically ill human patients.[8] A more recently developed noninvasive method of measuring Mg_i in humans uses sublingual epithelial cells and energy-dispersive x-ray analysis.[16] This method was found to correlate well with Mg_i measured from a surgically obtained atrial biopsy specimen in patients undergoing cardiopulmonary bypass, but it did not correlate with Mg_s.[16] This method of determination holds some promise for rapid and accurate measurement of Mg_i in human and veterinary patients.

A complication that may arise when muscle is used for determination of intracellular electrolyte concentrations on a dry-matter basis is the presence of fat and fascia in the tissue specimen. It has been found in our laboratory that increasing fat concentrations in tissue specimens will decrease the concentrations of all elements. It has specifically been found in our laboratory that sulfur varies indirectly and predictably with fat percentage in muscle specimens. Sulfur is a stable element in tissues that occurs primarily in amino acids and is not found in fat. In our study, attempts were made to remove all visible fat and fascia from the muscle specimens, and the results were standardized to sulfur content. Correcting to sulfur content eliminated the influence that excess fat in a muscle specimen would have on Mg_i.

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