Evaluation of the influence of S-adenosylmethionine on systemic and hepatic effects of prednisolone in dogs

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Objective—To evaluate the influence of a 1,4-butanedisulfonate stable salt of S-adenosylmethionine (SAMe) administered orally on clinicopathologic and hepatic effects induced by long-term administration of prednisolone in dogs.

Animals—12 healthy dogs.

Procedure—Following a pilot study (4 dogs), 2 groups of 4 dogs received prednisolone (2.2 mg/kg) orally once daily (84-day trial). One group received SAMe (20 mg/kg/d divided in 2 doses) for 42 days and then a placebo for 42 days; the other group received treatments in the reverse order. Before and during the trial, numerous variables were monitored, including serum total alkaline phosphatase (ALP) and glucocorticoid-induced ALP (G-ALP) activities, serum haptoglobin concentration, and total and oxidized glutathione (TGSH and GSSG) and thiobarbiturate-reacting substances (TBARS) concentrations in erythrocytes and liver tissue (days 0, 42, and 84). Hepatic specimens also were examined microscopically.

Results—The stable salt of SAMe was biologically available; plasma concentrations of SAMe or prednisolone were not affected by coadministration. Compared with baseline values, serum ALP and G-ALP activities and haptoglobin concentrations increased and erythrocyte GSSG and TBARS concentrations decreased with both treatments. Erythrocyte TGSH concentration decreased with the prednisolone-placebo treatment. Administration of SAMe appeared to conserve erythrocyte TGSH values and did not inhibit hepatocyte glycogen vacuolation but increased hepatic TGSH concentration and improved the hepatic tissue GSSG:TGSH ratio.

Conclusions and Clinical Relevance—In dogs, administration of 20 mg of SAMe/kg/d may mitigate the apparent pro-oxidant influences of prednisolone but did not block development of classic clinicopathologic or histologic features of vacuolar hepatopathy. (*Am J Vet Res* 2005;66:330–341)

S-adenosylmethionine (SAMe) is a nucleotide-like molecule that is synthesized from methionine and ATP by all living cells. Although distributed to almost all body tissues and fluids, SAMe has particular importance in hepatocytes, which influence the bulk of intermediary metabolism.[1–5] S-adenosylmethionine plays a pivotal role in 3 important biochemical pathways: transmethylation, transsulfuration, and aminopropylation. The transmethylation pathway transfers methyl groups from SAMe to a wide variety of molecules, including, but not limited to, phospholipids, neurotransmitters, nucleic acids, proteins, several intermediary metabolites (eg, l-carnitine, creatine, and polyunsaturated phosphatidylcholine), and many xenobiotics.[6,7] Methyl-group transfer enables molecular biotransformations essential for a myriad of metabolic reactions and maintenance of cell-membrane integrity, fluidity, and surface receptors; these effects influence cell adaptation to environmental changes and facilitate intercellular signaling. The transsulfuration pathway generates endogenous sulfur compounds, including cysteine and **glutathione (GSH)**, that are essential for management of cell redox status, detoxification and conjugation reactions, normal enzyme functions, protein-structural configurations, and gene expression.[8,9] S-adenosylmethionine has a major role in GSH synthesis as a substrate provider; the enabled production of GSH promotes antioxidant protection of synthetic pathway enzymes as well as a concentration-dependent, activating effect on cystathionine synthase (the rate-limiting enzyme in GSH synthesis).[10] The aminopropylation pathway yields polyamines and methylthioadenosine, which have important signaling and gene-transcription functions as well as anti-inflammatory effects.[8] Polyamines influence cell regeneration and repair through effects on DNA turnover, cell replication, and apoptotic pathways.

Many mechanistic studies have clarified essential and diverse roles of SAMe in health and disease at the molecular level, and most work has involved hepatocytes as a test system. Because the liver is a major site of systemic SAMe synthesis (metabolizing nearly 50% of dietary methionine, directly converting approx 80% of this to SAMe, and conducting 85% of the transmethylation reactions), this organ is a prime target for metabolic failure when SAMe is limited.[3] A major consequence of limited SAMe production in severe liver disease, among many detrimental effects, is reduced GSH availability.[7,11] Although liver injury can develop from diverse causes that often remain unknown, pathologic sequelae are predictable and commonly involve free-radical propagation that results in cell- and organelle-membrane damage that would be per-

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missively augmented by reduced GSH availability.[5,6,8–15] Recent determination of hepatic GSH content in dogs with naturally occurring liver disease confirmed that 44% of dogs with necroinflammatory disorders and 33% of dogs with vacuolar hepatopathy developed subnormal liver GSH concentrations.[16] Considering the central role of the liver in intermediary metabolism, conserving hepatocellular redox status has important implications for systemic health and metabolism and for recovery from disease. In patients with various liver disorders, treatment protocols intended to restore impaired hepatocyte function; enhance cell repair and regeneration; promote toxin neutralization or elimination; and attenuate inflammation, free radical accumulation, and cholestasis may broadly be of benefit. Thus, as has been proposed for humans, SAMe might contribute importantly to the management of veterinary patients with various liver disorders by providing similar protective influences.[17–23]

The high reactivity of natural SAMe limits its therapeutic usefulness. However, synthesis of a stable salt formulated in an enteric-coated tablet has allowed its use as a treatment.[2] Although hepatocellular uptake of SAMe remains controversial, incorporation of SAMe into the cell membrane and its ability to influence hepatocyte metabolism have been proven. Important regulatory functions of this molecule in hepatocytes have been defined,[8] and results of several clinical studies[17,18,21–23] in humans with liver disease indicate that SAMe may provide important therapeutic benefits.

Vacuolar hepatopathy is a disorder that is commonly encountered among dogs in clinical practice. In many instances, the underlying cause is either glucocorticoid administration or idiopathic overproduction of endogenous glucocorticoids or steroid sex hormones. The clinical syndrome associated with the glucocorticoid-induced vacuolar hepatopathy has been well described and modeled.[24–33]

The purpose of the study reported here was to evaluate the influence of an orally administered 1,4-butanedisulfonate stable salt of SAMe[a] on the clinicopathologic and hepatic effects induced by long-term oral administration of prednisolone in dogs. A prospective double-blind, placebo-controlled crossover design was used to test the hypotheses that SAMe could attenuate adverse systemic and hepatic effects associated with prednisolone administered orally in high doses to dogs during an 84-day period.

Materials and Methods

Twelve healthy adult mixed-breed dogs (6 sexually intact females and 6 sexually intact males) that were 2 to 7 years of age and weighed 12.3 to 16.4 kg were included in the study. Animal care was in full compliance with the laboratory animal use and housing protocols of Cornell University, and the experimental protocol was approved by the university's Institutional Animal Care and Use Committee. The health status of each dog was ascertained on the basis of findings of physical examination and routine laboratory health assessments, including a CBC, serum biochemical profile (including measurement of concentrations of total protein, albumin, BUN, creatinine, total bilirubin, glucose, cholesterol, sodium, potassium, chloride, calcium, phosphate, and pre- and postprandial serum bile acids and activities of alanine aminotransferase, aspartate aminotransferase, **alkaline phosphatase** [**ALP**], and **γ-glutamyltransferase** [**GGT**]), urinalysis, occult heartworm test, and histologic evaluation of a liver biopsy specimen. All dogs were vaccinated currently against infectious viral diseases and were free of enteric parasites. Dogs were group housed with 2 to 4 dogs of the same sex/room, fed a commercial dog chow for 2 months preceding and throughout the study, and provided water free choice at all times. During the study, food was freely available from 10 AM to 6 PM daily.

Pilot study—An initial pilot study was conducted with 4 sexually intact adult dogs (2 females and 2 males) to investigate for pharmacologic interactions resulting from co-administration of SAMe and prednisolone. A double-blind, placebo-controlled crossover design study was used. All dogs received a loading dose of prednisolone at 2.2 mg/kg for 3 consecutive days, followed by a maintenance dose at 1.1 mg/kg, PO, every 24 hours (administered in the morning after food had been withheld for 12 hours). The SAMe[a] was administered in 2 doses to provide at least 20 mg of SAMe/kg/d; because tablets were coated, tablets were not divided into portions. Dogs were allocated to 1 of 2 groups (1 male and 1 female/group) and received either SAMe and prednisolone (SAMe-prednisolone treatment) or placebo and prednisolone (placebo-prednisolone treatment). Dogs received either SAMe or identical-appearing, enteric-coated placebo tablets in the morning after food had been withheld for 12 hours overnight and in the evening after any remaining food was removed. On day 21, each group received the alternate treatment, which was continued for 21 days. The total daily dose of SAMe was given after baseline samples were obtained on days when pharmacokinetic studies were conducted. On days 0 (ie, 1 day before the study commenced), 21, and 42, blood samples were obtained before (time 0) and at 1, 2, 4, 6, 8, 10, and 24 hours after dosing; blood was collected in evacuated tubes containing lithium heparin[b] for determination of plasma SAMe and prednisolone concentrations. Analysis of data from this study was completed before initiation of the clinical trial. On the basis of modest hematologic and serum biochemical changes over 42 days, a higher dose of prednisolone was used in the clinical trial.

Clinical trial—After analysis of the findings of the pilot study, a trial was done with 8 different sexually intact adult dogs (4 females and 4 males). These dogs were given a loading dose of prednisolone (4.4 mg/kg) and maintained for 84 days on 2.2 mg of prednisolone/kg administered orally once in the morning after food had been withheld for 12 hours. A double-blind, placebo-controlled crossover design was again used in this trial. Dogs were allocated to 1 of 2 groups by lottery so that there were equal numbers of male and female dogs in each group; groups were given either the SAMe-prednisolone or placebo-prednisolone treatment. On day 43, each group received the alternate treatment, which was continued for 42 days.

All blood and tissue specimens were collected after food had been withheld for 12 hours before SAMe, placebo, or prednisolone administration. Blood and urine samples were collected on days 0 (ie, 1 day before the study commenced), 14, 28, 42, 56, 70, and 84 for a CBC, serum biochemical profile, assessment of glucocorticoid ALP-isoenzyme activity, measurement of serum haptoglobin and bile acids concentrations, and urinalysis. To perform the CBCs, blood was collected into evacuated tubes containing EDTA[c]; hematologic evaluation was completed by use of automated procedures[d] with manual assessment of differential cell counts and cell morphology. The counting instrument was calibrated for canine blood cells and was used to determine PCV, hemoglo-

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bin concentration, RBC and WBC counts, **mean corpuscular volume (MCV)**, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and **red cell distribution width (RDW [%])**. Serum biochemical profiles and haptoglobin concentration determinations were completed by use of automated[e] procedures, as previously described.[34] The glucocorticoid-induced ALP-isoenzyme activity was determined by use of the levamisole-inhibition assay on the automated system.[35] After food had been withheld for 12 hours and 2 hours after food was provided, serum bile acids concentration was determined by use of a linked enzymatic reaction on an automated system.[36,e,f] The highest serum bile acids concentration of the paired samples was used for statistical comparisons. Assessment of the percentage retention and plasma clearance of indocyanine green was completed on days 0, 14, 42, 56, and 84. The indocyanine green (in freeze-dried form)[g] was reconstituted to a 0.5% solution with sterile saline (0.9% NaCl) solution immediately prior to use and administered at a dose of 1.5 mg/kg to each dog via rapid IV bolus injection. Blood was collected into evacuated tubes containing lithium heparin[b] before indocyanine green administration (time 0) and thereafter from a different vein at 3, 6, 9, 12, 15, and 30 minutes. The plasma concentration of indocyanine green was measured directly by use of a spectrophotometer at 805 nm by comparison of sample absorbance to a standard curve.[37] The extrapolated concentration of plasma indocyanine green at time 0 was derived from the clearance analysis and was used to calculate the percentage retention at 30 minutes. Plasma clearance rate was calculated on the basis of the indocyanine green concentrations in plasma samples obtained within the first 15 minutes of administration of indocyanine green.

Blood was collected into tubes containing preservative-free sodium heparin and transported in a melting ice bath for immediate determination of concentrations of **total GSH (TGSH)**, **reduced GSH (RGSH)**, **oxidized glutathione (GSSG)**, and **thiobarbiturate-reacting substances (TBARS)** in erythrocytes on days 0, 28, 42, 70, and 84. The TGSH concentration was determined by use of a modification of the procedure described by Prins and Loos.[38] Concentrations of GSSG were determined by use of a modification of the procedure described by Beutler et al.[39] Standard solutions prepared with known concentrations of GSH[h] or GSSG[i] were assayed simultaneously. Concentrations of total GSH and GSSG in blood were normalized by use of the sample PCV and expressed as milligrams per deciliter of RBCs. The concentration of TBARS was measured in blood washed in cold isotonic saline solution and suspended in a buffer containing butylated hydroxytoluene[j] (to minimize membrane oxidation during processing) and thiobarbituric acid, according to the method of Beuge and Aust.[40] Standards were prepared via hydrolysis of 1,1,3,3-tetraethoxypropane, and TBARS concentration was normalized by use of the sample PCV and expressed as micromole per liter of RBCs.[41]

Determination of plasma SAMe and prednisolone concentrations—In the pilot study, plasma SAMe and prednisolone concentrations were determined on days 0, 21, and 42 at 0, 1, 2, 4, 6, 8, 10, and 24 hours after dosing. To assess the 12-hour trough plasma SAMe and prednisolone concentrations during the clinical trial, blood samples were collected on day 0 and thereafter at 12 hours after administration of the evening SAMe dose but before morning treatment on days 7, 14, 21, 28, and 42 from all dogs in each group. Blood samples for SAMe and prednisolone[k] analyses were transported on ice and centrifuged promptly at –20°C; plasma samples were kept frozen at –80°C until thawed for batched analyses.

Plasma for SAMe determination was deproteinized by use of 1 volume of ice-cold, 0.8M perchloric acid. The SAMe analyses were completed by use of a validated (S,S-adenosylmethione), quality-controlled, high-pressure liquid chromatography technique, as previously described.[42,43] Separation peaks were confirmed via alkaline hydrolysis of SAMe in plasma (resulting in the quick disappearance of the SAMe), enzymatic synthesis of SAMe (through the action of ATP-methionine methyltransferase), and spiking of plasma samples with appropriate standards. The method was proven to be linear for concentrations of SAMe as high as 3.0 mg/L with a sensitivity of < 5 µg/L. Recovery of SAMe spiked into human plasma was compared with direct injection of standards onto the analytical column. Mean recoveries (5 samples included in each analysis) of 99.6%, 97.8%, and 101% were found at 29, 143, and 1,430 ng/mL, respectively. Assay accuracy was determined by calculating the percentage of detected SAMe versus the actual concentration of SAMe in human plasma spiked with 32 to 2,300 ng/mL of SAMe; values ranged from 94.9% to 99.5%. The intra-assay coefficient of variation in SAMe-spiked plasma (independent from calibration curves) was ≤ 5.0% in batched human plasma pools that were analyzed 5 times each at baseline concentration (20 ng/mL) and baseline concentration spiked with 32, 893, and 2,300 ng/mL of SAMe. The interassay coefficient of variation for samples from these pools was < 7.2% (n = 15). Mean ± SD accuracy of the analytic method in canine plasma (1,200 ng/mL; n = 21) was 99.9 ± 3.7%. Eight repeated measurements of a pooled canine plasma sample yielded an interassay coefficient of variation of 8.9%. Concentration of SAMe in unknown samples was determined via linear regression analysis by use of the concentration-to-peak height ratio method.

Plasma prednisolone concentrations were determined as described by Hirata et al[44] with certain procedural modifications. Plasma was extracted by use of 50-mg, solid-phase extraction cartridges[l] preconditioned with methanol (0.5 mL) and deionized water (0.5 mL). After plasma (0.5 mL) was added, the cartridge was washed with water; excess water was then removed by use of a vacuum system. The sample was eluted with methanol (0.5 mL), evaporated under nitrogen at 50°C, and reconstituted in 0.2 mL of mobile phase A (isopropanol-0.05M acetate buffer; pH, 4.7; vol/vol, 10/90) for injection onto the high-pressure liquid chromatographic column. Mobile phase B was isopropanol-0.05M acetate buffer (pH, 4.7; vol/vol, 30/70). Initial gradient conditions were set at 90% mobile phase A and 10% mobile phase B for 5 minutes; thereafter, mobile phase A was changed from 90% to 40% over 15 minutes (linear gradient), with the final condition held for 5 minutes and then returned to the initial condition for 5 minutes before the next sample injection. Flow rate was constant at 1 mL/min, and temperature was maintained at 40°C during the entire gradient period; total run time was 25 minutes. A reverse-phase analytical column[m] (5 µm; 25 × 4.6-cm internal diameter) and a radial compression guard column unit[n] were used for separation and detection. Standard curves prepared in pooled canine plasma over a concentration range of 50 to 600 ng/mL were proven to be linear (r^2 = 0.99). Quality-control samples (200 ng/mL) prepared in canine plasma and extracted in duplicate with each of 5 sets of samples yielded an interassay coefficient of variation of 14.8%. Plasma concentration of prednisolone in unknown samples was determined via linear regression analysis by use of the concentration-to-peak height ratio method.

Collection and histologic evaluation of liver biopsy specimens—Liver biopsy specimens were collected from the study dogs at baseline and on day 42 of each treatment during the clinical trial (ie, on days 42 and 84 of the trial); specimens were also obtained from 5 clinically healthy dogs used as controls in an unrelated research project (animal care was in full compliance with the laboratory animal use and housing protocols of Cornell University and the experimental pro-

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tocol was approved by the university's Institutional Animal Care and Use Committee). For liver biopsy collection, all dogs received butorphanol (0.05 mg/kg) and glycopyrrolate (0.1 mg/kg) 30 minutes before induction of anesthesia with isoflurane administered via a mask. Anesthesia was maintained with isoflurane. At least 7 liver biopsy specimens (from at least 4 different liver lobes) were collected via a laparoscopic technique; specimens weighed 60 to 160 mg. Postoperatively, dogs were administered butorphanol (0.1 mg/kg, IM or IV) as needed to ameliorate discomfort; in no instance was this treatment required beyond 24 hours. Liver specimens were fixed in neutral-buffered 10% formalin for routine histologic sectioning and staining with H&E. Two biopsy specimens were fixed in absolute alcohol for **periodic acid-Schiff (PAS)** staining of glycogen, with and without diastase (amylase). Other liver tissue specimens were snap-frozen in liquid nitrogen and transferred to a freezer (–80°C) until thawed for analysis of tissue concentrations of TGSH, RGSH, GSSG, TBARS, glycogen, total protein, and DNA.

Liver specimens were evaluated histologically; on a 10X-magnified field, the degree and distribution of vacuolar transformation were assessed by use of a numerical grading system: 1 = no vacuolation, 2 = ≤ 25% of hepatocytes were vacuolated, 3 = 26% to 50% of hepatocytes were vacuolated, and 4 = ≥ 51% of hepatocytes were vacuolated. Also, a score was assigned to designate zonal distribution of vacuolar change: 1 = periportal or localized to zone 1; 2 = midzonal or localized to zone 2; 3 = perivenular, periacinar, or localized to zone 3; and 4 = diffuse vacuolation. Hepatic tissue was inspected microscopically for cell binucleation, which would invalidate DNA measurements intended to normalize tissue measurements to hepatocyte numbers. Abnormal glycogen accumulation was defined by the presence of diastase-resistant, PAS-stain retention in the hepatocellular cytosol, as compared qualitatively with results of PAS-staining of liver tissue obtained from 5 clinically healthy dogs: 1 = no difference from extent of PAS-stain retention detected in tissue from clinically normal dogs, 2 = mild PAS-stain retention (in ≤ 25% of hepatocytes), 3 = moderate PAS-stain retention (in 26% to 50% of hepatocytes), and 4 = marked PAS-stain retention (in ≥ 51% of hepatocytes).

Morphometric assessment of hepatocytes was used to quantify the effect of treatment on cell size (cell area and circumference). By use of a flatbed scanner, an image (400X) of routinely fixed tissues stained with H&E was captured and manipulated with National Institutes of Health imaging freeware to determine cell size. Three sections containing representative areas of hepatic parenchymal vacuolation that included at least 50 intact hepatocytes were used for measurements. One individual (SAC) delineated cell margins of only hepatocytes that had a nuclear profile.

Liver tissue assays—Liver tissue concentrations of TGSH and GSSG were determined in duplicate by use of a modification of the recycling reaction and kinetic method,[45] which has been used by this group in another study.[16] Liver tissue RGSH values were then calculated. The recycling reaction measures all GSH moieties (ie, RGSH and GSSG). Determination of TGSH after extraction of RGSH with 2-vinylpyridine[o] permitted measurement of GSSG. Standards (RGSH[h] and GSSG[i]) prepared in the same assay buffer were treated with 5% (wt/vol) trichloroacetic acid and processed through the same extraction procedures. A portion of liver tissue homogenate was retained for determination of protein and DNA concentrations.

Liver tissue TBARS concentration was measured by use of the method of Uchiyama and Mihara.[46] Liver tissue was homogenized in a melting ice bath with ice-cold phosphate buffer solution (0.01M; wt/vol, 1 to 9; pH, 7.4) containing butylated hydroxytoluene[j] and protein precipitated by use of 5% (wt/vol) trichloroacetic acid. The supernatant was combined with 1% phosphoric acid (vol/vol) and 0.6% thiobarbituric acid solution (wt/vol) and heated in a boiling water bath for 45 minutes; malonaldehyde concentration (representing TBARS concentration) in the supernatant was determined spectrophotometrically by assessment of the difference in optical density at 535 and 520 nm. Tetramethoxypropane (malondialdehyde) was measured as an external standard, as described.[41]

Glycogen concentration in liver tissue was measured by use of a phenol-sulfuric acid technique appropriate for microanalyses of glycogen in tissues. Samples were assayed in triplicate, and solutions of known glycogen concentrations were measured as external standards.

Tissue concentrations of TGSH, RGSH, GSSG, and glycogen were expressed on the basis of liver tissue wet weight and tissue protein (mg) and DNA (µg) concentrations because of the concern that cell expansion with glycogen or other accumulated material associated with hepatocellular vacuolation would alter tissue wet weight and cell size, thereby influencing outcome analyses. Tissue protein was determined by use of the Bradford method.[48] External standards were prepared with known quantities of bovine serum albumin[p] to correct for binding characteristics of the dye in the buffer systems used for tissue processing. Tissue DNA concentrations were determined by use of a modification of the fluorescent method described by Downs and Wilfinger,[49] which is based on the reaction between the bisbenzimidazole reagent[q] and DNA. A DNA standard was prepared from highly polymerized calf thymus DNA[r]; standards were measured before and after evaluation of sample fluorescence to correct for any decay in fluorescence over time.

Statistical evaluations—Because a lower dose of prednisolone was used in the pilot study, compared with that used in the clinical trial, only the plasma SAMe and prednisolone concentrations used in the clinical trial were reported and statistically analyzed. All samples were analyzed and data evaluated without investigator knowledge of group designations. Data were examined for normal distribution by use of box-and-whisker plots and descriptive statistics. Nonparametric analyses were designated for most analyses except pharmacologic data and percentage changes of RBC and tissue variables. Data evaluated by use of nonparametric measures are reported as median, 95% **confidence interval (CI)**, and range; data evaluated by use of parametric measures are reported as mean ± SD or SEM, as indicated.

Data were grouped into SAMe-prednisolone or placebo-prednisolone treatment. When statistical comparisons of treatments at baseline and identical treatments before and after protocol crossover did not identify significant differences, 28- and 42-day values for identical treatments were pooled. Significant differences were determined by comparison of day 0 (baseline) to treatment days and by comparison of treatments. Pharmacologic data obtained during the pilot study were evaluated by calculating the **area under the curve (AUC)** of plasma SAMe and prednisolone concentrations by use of the trapezoidal rule. The AUC (pilot study) and 12-hour trough plasma SAMe and prednisolone concentrations (clinical study) were compared between days on which samples were collected and groups.

Differences in nonparametric data within a treatment group (baseline vs treatment day) were tested by use of the Wilcoxon signed rank test, and between treatments were tested by use of the rank sum (2-sample Mann-Whitney) test; an α of < 0.05 was applied with a 2-tailed *P* value. Differences in parametric data (AUCs for plasma prednisolone and SAMe concentrations and percentage changes in erythrocyte TGSH and TBARS concentrations and liver TGSH concentration) were tested by use of a 1-way ANOVA, with the Fisher least significant difference test applied with an α of 0.05.

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Results

In the pilot study, plasma SAMe AUC was significantly ($P < 0.001$) greater in the SAMe-prednisolone treatment group than in the placebo-prednisolone treatment group at day 0 (mean ± SD, 11,561 ± 5,386 ng/mL vs 551 ± 148 ng/mL) and after 21 days of treatment (9,957 ± 4,482 ng/mL vs 678 ± 125 ng/mL). There were no significant differences in the plasma SAMe AUC between days 0 and 21 within either treatment group. Peak plasma SAMe values occurred in individual dogs from 1 to 10 hours after administration of SAMe, and most dogs developed peak values within 1 to 4 hours; this likely reflects differences in enteric transit but also might represent differences in SAMe tissue uptake or its biotransformation to metabolites. A significantly ($P < 0.001$) higher trough plasma concentration of SAMe (12-hour trough of the divided daily SAMe dose) was found in the dogs receiving the SAMe-prednisolone treatment, compared with day-0 measurements and the placebo-prednisolone treatment (**Figure 1**). For both treatments, plasma prednisolone concentration (assessed as AUC) on day 0 in the pilot study significantly exceeded concentrations on subsequent sampling days because of the loading dose administered during the initial 3 treatment days (2.2 mg/kg/d). In dogs receiving the SAMe-prednisolone and placebo-prednisolone treatments, the initial plasma prednisolone concentration (mean ± SD) was 6,863 ± 2,959 ng/mL and 6,807 ± 2,222 ng/mL, respectively. On day 21, these values were 2,882 ± 1,481 ng/mL and 2,863 ± 622 ng/mL, respectively, and on day 42, these values were 2,446 ± 224 ng/mL and 2,199 ± 103 ng/mL, respectively. There were no significant differences in plasma prednisolone AUC values between data points or treatments. Trough plasma prednisolone concentrations (at time 0, representing a 24-hour trough prednisolone concentration) were undetectable (< 50 ng/mL).

For each treatment, PCV values were significantly lower than baseline values throughout the study; nevertheless, except for 2 dogs (1 dog in each group) that had PCV values of 36% and 37%, respectively, on day 14, PCV values remained within the reference range (39% to 57%). These minimal reductions in PCV were attributed to blood sampling. There were no significant changes in values of MCV or RDW between baseline and subsequent data points. All dogs developed eosinopenia within 2 weeks of treatment initiation, which was sustained throughout the study. There were no other significant differences in hematologic variables between treatments.

There were few significant changes in serum biochemical variables within or between treatments. Compared with day-0 values, serum ALP activity was significantly increased in all dogs by day 14 and thereafter remained higher than baseline values. However, few dogs had serum ALP activity that exceeded the upper reference limit (ie, > 112 U/L). The magnitude of the increase in serum ALP activity was not significantly different between treatments and was not more than 2.3 times greater than the baseline value on an individual basis. Compared with day-0 data, the serum glucocorticoid-induced ALP-isoenzyme activity was significantly increased in dogs receiving either treatment on day 28 and in dogs receiving the placebo-prednisolone treatment on day 42. Values of serum glucocorticoid ALP-isoenzyme activity exceeded the upper limit of the reference range (ie, > 20 U/L) in only 1 dog receiving placebo-prednisolone on day 28. There were no significant differences in serum glucocorticoid ALP-isoenzyme activity between treatments at any time point. The magnitude of the increase in serum GGT activity paralleled changes in serum ALP activity. At any time point, there were no significant differences in serum GGT activity between treatments and values remained within the reference range (reference range, 0 to 11 U/L) in all dogs. Although serum cholesterol concentrations gradually increased through day 42, median cholesterol values were not significantly increased, compared with day-0 values, and were not significantly different between treatments. During the interval from days 0 to 7 in each study group, serum haptoglobin concentrations increased significantly (to 3 times the day-0 values) and this increase was sustained throughout the study; serum haptoglobin con-

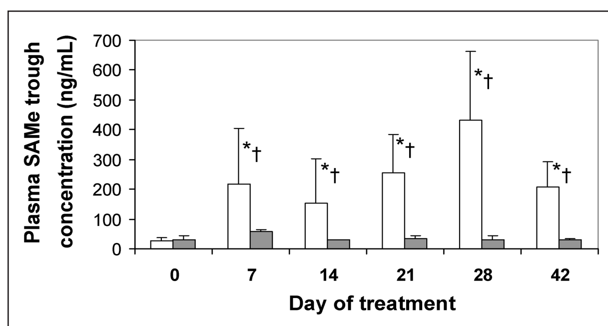

Figure 1—Mean ± SD 12-hour trough plasma S-adenosylmethionine (SAMe) concentrations in 8 dogs before administration of treatment (day 0) and at intervals until day 42 of treatment (pooled data) with SAMe and prednisolone (open bars) or placebo and prednisolone (solid bars) in an 84-day crossover study. *Value significantly ($P < 0.001$) different between treatments. †Value significantly ($P < 0.001$) different from day-0 value.

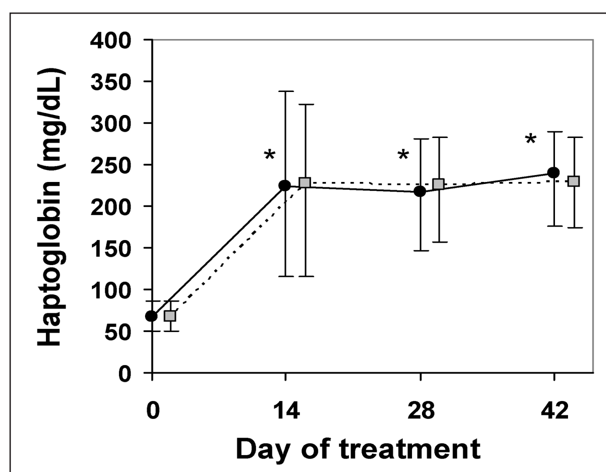

Figure 2—Median serum haptoglobin concentrations in 8 dogs before administration of treatment (day 0) and at intervals until day 42 of treatment with SAMe and prednisolone (circles) or placebo and prednisolone (squares) in an 84-day crossover study. Bars represent the 95% confidence interval. *Values for each treatment at this data point were significantly ($P < 0.03$) different from the day-0 value.

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Table 1—Serum concentration of erythrocyte total glutathione (TGSH) and oxidized glutathione (GSSG), GSSG:TSGH ratio as a percentage, and serum concentration of thiobarbiturate-reacting substances (TBARS) in 8 dogs before administration of treatment (day 0) and at days 28 and 42 of treatment with S-adenosylmethionine and prednisolone (SAMe-prednisolone) or placebo and prednisolone (placebo-prednisolone) in an 84-day crossover study.

Time point	Erythrocyte TGSH (mg/dL)		Erythrocyte GSSG (mg/dL)		GSSG:TGSH ratio (%)		Erythrocyte TBARS (μmol/L)	
	SAMe-prednisolone	Placebo-prednisolone	SAMe-prednisolone	Placebo-prednisolone	SAMe-prednisolone	Placebo-prednisolone	SAMe-prednisolone	Placebo-prednisolone
Day 0								
Median (range)	65.1 (46.6–85.6)		1.1 (0.8–1.4)		1.6 (1.2–2.3)		11.3 (4.4–13.3)	
95% CI	54.8–76.5		0.9–1.2		1.3–2.0		7.4–13.0	
Day 28								
Median (range)	61.7 (53.7–71.4)	54.2 (41.2–72.2)	0.05* (0–1.0)	0.08* (0–0.3)	0.07* (0–1.8)	0.08* (0–0.8)	4.8* (2.5–11.0)	5.0* (2.1–7.3)
95% CI	56.2–65.6	46.3–63.2	0–0.4	0–0.2	0–0.9	0–0.4	2.8–7.6	2.9–6.3
Day 42								
Median (range)	61.7 (53.6–91.4)	55.5 (46.6–65.6)	0* (0–0.6)	0.3* (0–0.6)	0* (0–0.8)	0.3* (0–1.9)	4.2* (3.9–8.7)	5.0* (4.0–6.5)
	55.7–77.7	56.1–62.5	0–0.2	0–0.6	0.4	0–1.1	3.6–6.8	4.3–5.6

*Value significantly ($P < 0.05$) different from day-0 value.
CI = Confidence interval.

centrations exceeded the upper limit of the reference range (reference range, 28 to 128 mg/dL; **Figure 2**). There were no significant differences in serum haptoglobin concentration between treatments. Although the serum bile acids concentration was significantly increased in dogs receiving placebo-prednisolone on days 28 and 42, compared with the day-0 concentration, values remained within the reference range (reference range, 0 to 25 µmol/L) and changes were not considered clinically important. Plasma indocyanine green retention and clearance values remained within the reference ranges (reference ranges, 14.7 ± 5.0% and 3.7 ± 0.7 mL/min/kg, respectively) in dogs receiving either treatment and were not significantly different between treatments or sampling days.

On inspection of the data, a decrease in median blood TGSH concentration from day 0 occurred with each treatment, but the change from baseline was not significant (**Table 1**). The erythrocyte GSSG concentration, GSSG:TGSH ratio, and TBARS concentration were significantly decreased at days 28 and 42, compared with day-0 data, for each treatment. The percentage change in erythrocyte TGSH concentration by day 42 was significantly different between treatments (percentage change calculated from the difference between day-42 measurements and day-0 values; **Figure 3**); SAMe-prednisolone treatment appeared to conserve TGSH concentrations. However, a similar percentage decline in erythrocyte TBARS concentration was detected with each treatment (44.6% in dogs receiving SAMe-prednisolone treatment and 44.9% in dogs receiving the placebo-prednisolone treatment) at day 42.

Histologic examination of liver tissue revealed progressive hepatocellular vacuolation associated with increased variation in cell size during either treatment (**Table 2**). Histologically, liver biopsy specimens obtained on day 0 had mild vacuolar changes (3 dogs); 2 dogs had focal vacuolation in zone 2, and 1 dog had randomly distributed focal hepatocyte vacuolation. On day 42, varying degrees of vacuolar change were detected in each dog; 4 dogs (2 were receiving the SAMe-prednisolone treatment, and 2 were receiving the placebo-

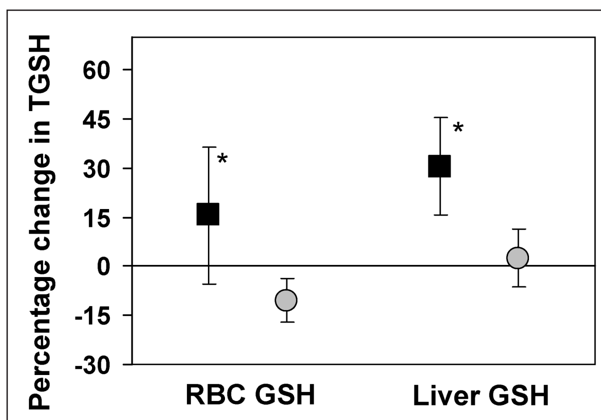

Figure 3—Mean ± SEM percentage change in erythrocyte and liver total glutathione (TGSH) concentrations in 8 dogs after 42 days of treatment with SAMe and prednisolone (squares) or placebo and prednisolone (circles) in an 84-day crossover study. *Value significantly ($P < 0.03$) different between treatments.

prednisolone treatment) had a marked, diffuse vacuolar change involving zones 1 and 2. Progressive hepatocellular vacuolation in each dog during the study reflected the chronicity of prednisolone administration. By day 84 of prednisolone treatment, all dogs had developed a marked, diffuse or zonal (zones 1 and 2) vacuolar hepatopathy. For both groups of dogs, histologic scores for vacuolation at days 42 and 84 were each significantly different from those obtained on day 0; however, there were no significant differences in histologic scores between treatments. In both groups of dogs, results of PAS staining qualitatively confirmed increased hepatocellular glycogen concentrations in liver biopsy specimens obtained at days 42 and 84, compared with day-0 findings, but there was no significant difference in glycogen accumulation between groups as determined by use of PAS staining scores. None of the liver specimens from any of the dogs had histologic changes suggestive of a toxic reaction to SAMe, and hepatocyte binucleation was not detected microscopically. Morphometric analyses confirmed a significant increase in hepatocyte size (cell area and circumference) after 42 days of either treatment, compared with day-0 data. However, cell size was

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not significantly different between treatments. Furthermore, the cell area increased by a mean of 78 ± 32% and 64 ± 33% after SAMe-prednisolone and placebo-prednisolone treatments, respectively; the percentage change in hepatocyte dimensions was not significantly different between treatments.

Table 2—Hepatocyte circumference and perimeter measurements in 8 dogs before administration of treatment (day 0) and after 42 days of treatment with SAMe-prednisolone or placebo-prednisolone in an 84-day crossover study.

Variable	Day 0	SAMe-prednisolone (42 d)	Placebo-prednisolone (42 d)
Median (range) hepatocyte area (μm^2)	442 (382–482)	801* (508–867)	694* (576–868)
95% CI	411–467	675–864	606–820
Median (range) hepatocyte perimeter (μm)	85 (77–87)	111* (97–123)	111* (95–120)
95% CI	80–86	105–118	100–118

*Value significantly ($P < 0.01$) different from day-0 value.
See Table 1 for remainder of key.

There were no significant differences in liver protein or DNA concentrations between treatments (**Table 3**). Although significant increases in liver tissue glycogen concentration were detected with each treatment, compared with baseline values, there were no significant differences between treatments. The percentage change in liver tissue glycogen concentration also was not significantly different between treatments (liver tissue glycogen concentration increased by a median of 123% [range, 13% to 636%] in association with the SAMe-prednisolone treatment and by a median of 108% [range, 11% to 609%] in association with the placebo-prednisolone treatment).

Although liver TGSH and RGSH concentrations increased from day 0 with each treatment (on inspection of data), only changes associated with the SAMe-prednisolone treatment were significant (**Table 4**). This corresponded with a significantly higher percentage increase in hepatic TGSH concentration and a significantly lower tissue GSSG:TGSH ratio, compared with day-0 data. These changes suggest improved tissue redox status with GSH retained in its reduced form

Table 3—Hepatic tissue concentrations of DNA, protein, and glycogen in 8 dogs before administration of treatment (day 0) and after 42 days of treatment with SAMe-prednisolone or placebo-prednisolone in an 84-day crossover study.

Variable	Day 0	SAMe-prednisolone (42 d)	Placebo-prednisolone (42 d)
Median (range) DNA concentration (μg/g of liver tissue)	3,513 (2,587–3,840)	3,420 (2,794–4,763)	2,994 (2,108–4,869)
95% CI	2,965–3,744	2,962–4,099	2,430–4,059
Median (range) protein concentration (mg/g of liver tissue)	128.6 (90.3–147.8)	139.3 (118.5–157.4)	133.2 (118.7–149.3)
95% CI	109.1–142.6	127.3–149.0	127.3–145.3
Median (range) glycogen concentration (mg/g of liver tissue)	25.7 (9.3–38.5)	53.3* (13.6–75.6)	50.7* (24.0–77.2)
95% CI	18.0–32.5	35.0–67.2	37.8–64.8
Median (range) glycogen concentration (μg/mg of liver tissue protein)	209.6 (66.1–285.4)	378.8* (114.9–564.8)	383.1* (160.6–649.9)
95% CI	143.4–269.1	253.0–479.3	265.3–501.6
Median (range) glycogen concentration (μg/μg of liver tissue DNA)	8.7 (2.6–10.2)	16.2* (4.9–26.8)	17.9* (8.1–25.6)
95% CI	5.4–9.9	9.1–20.8	11.5–22.1

*Value significantly ($P < 0.04$) different from day-0 value.
See Table 1 for remainder of key.

Table 4—Hepatic tissue concentrations of TGSH, reduced glutathione (RGSH), and GSSG and the GSSG:TSGH ratio as a percentage in 8 dogs before administration of treatment (day 0) and after 42 days of treatment with SAMe-prednisolone or placebo-prednisolone in an 84-day crossover study.

	Day 0		SAMe-prednisolone(42 d)		Placebo-prednisolone(42 d)	
Variable	Median (range)	95% CI	Median (range)	95% CI	Median (range)	95% CI
TGSH (μmol/g of liver)	2.2 (1.5–2.9)	1.8–2.5	2.9* (2.4–3.6)	2.6–3.3	2.7 (1.9–3.0)	2.1–2.9
TGSH (nmol/μg of DNA)	067 (0.44–0.86)	0.53–0.78	0.90* (0.51–1.27)	0.66–1.09	0.79 (0.39–1.41)	0.58–1.18
TGSH (nmol/mg of protein)	16.5 (11.8–24.2)	14.2–20.7	22.1* (17.3–25.7)	19.1–23.8	20.1 (14.4–25.0)	15.6–22.5
RGSH (μmol/g of liver)	2.0 (1.3–2.7)	1.6–2.3	2.7* (2.2–3.4)	2.4–3.1	2.5 (1.7–2.8)	2.0–2.8
RGSH (nmol/μg of DNA)	0.61 (0.38–0.80)	0.53–0.78	0.84 (0.46–1.20)	0.66–1.09	0.72 (0.35–1.32)	0.58–1.18
RGSH (nmol/mg of protein)	15.0 (10.3–22.4)	14.2–20.7	20.6* (15.4–24.2)	19.1–23.8	18.7 (13.0–23.3)	15.6–22.5
GSSG (nmol/g of liver)	82 (87–10)	92–101	94 (87–129)	87–112	99 (85–100)	90–100
GSSG (nmol/μg of DNA)	0.03 (0.03–0.4)	0.03–0.04	0.06 (0.02–0.04)	0.03–0.04	0.03 (0.02–0.04)	0.03–0.04
GSSG (nmol/mg of protein)	0.8 (0.6–1.1)	0.7–0.9	0.7 (0.6–0.9)	0.7–0.9	0.7 (0.7–0.9)	0.7–0.8
GSSG:TGSH ratio (%)	4.6 (3.6–6.4)	3.8–5.4	3.2* (2.6–5.3)	2.7-4.1	4.1(2.9–5.1)	3.2–4.6

*Value significantly ($P \leq 0.05$) different from day-0 value.
See Table 1 for remainder of key.

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because there was no change in tissue GSSG concentrations. Liver tissue TBARS concentrations were very low to undetectable on day 0 and after 42 days of each treatment in both groups.

Discussion

Pharmacologic findings of the present study indicate that the SAMe in the enteric-coated 1,4-butanedisulfonate salt form[a] is biologically available via oral administration and suggest that long-term coadministration of SAMe with prednisolone does not influence bioavailability or metabolism of either substance. Consistent with previous work[s] in dogs, we detected increased plasma SAMe concentrations within 1 to 10 hours of oral administration (usually with a well-defined peak within 1 to 4 hours) in animals from which food had been withheld overnight. The short duration of peak plasma SAMe concentrations is consistent with the reported short half-life of SAMe in dogs.[2,s] Dosing strategies (dosing in the absence of feeding) were designed to avoid delayed absorption and reduced bioavailability (reduced 4- to 10-fold) previously detected in dogs from which food had not been witheld.[2,s] Dividing the daily dose of SAMe into 2 treatments maintained measurable trough concentrations of SAMe 12 hours after dosing, likely reflecting a slow enteric uptake of SAMe from the coated tablets. In our study, enteric-coated tablets were used because in this form, SAMe has increased bioavailability, compared with that of the nonenteric-coated product, resulting in dose-related, peak plasma SAMe concentrations.[2] Although tissue dispersal of SAMe was not determined in the present study, finding significantly increased TGSH concentrations in the liver tissue of SAMe-treated dogs supports its bioavailability as a thiol substrate for the liver. As anticipated from results of previous investigations in dogs, we did not detect any adverse effects in dogs after administration of SAMe at a dose of 20 mg/kg.[2,s]

Vacuolar hepatopathy is a common hepatobiliary syndrome encountered in dogs in clinical practice.[24] Although it can be induced by administration of glucocorticoids via oral, parenteral, or topical routes, individual response to glucocorticoid treatment is highly variable. The marked differences in response are accounted for by individual variation as well as by different dosing regimens, routes of drug administration, and the specific glucocorticoid administered. The common clinical diagnosis of vacuolar hepatopathy in dogs that have not received exogenous glucocorticoids and do not have classic signs of hyperadrenocorticism suggests that this hepatopathy may reflect chronic stress of illness, infectious or inflammatory mediator release, or other hyperplastic adrenal disorders (eg, involving sex hormones). The physiologic curiosity presented by the unique susceptibility of dogs to this syndrome and its common clinical presentation has prompted numerous studies[25–33] to be undertaken to characterize its clinical, biochemical, and histologic features. In dogs, hepatic histologic changes are known to precede development of clinicopathologic changes that typify glucocorticoid or vacuolar hepatopathy. Thus, development of diffuse hepatocellular vacuolation in the absence of marked biochemical perturbations in dogs, as observed in the present study, is not unusual.

The dose of prednisolone investigated in our study was predicated by doses used in other investigations of vacuolar hepatopathy in dogs as well as by our desire to use a clinically relevant dose known to induce vacuolar liver lesions in canine clinical patients treated for immune-mediated and inflammatory disorders. Findings of prior evaluation[27] of a spectrum of high oral prednisolone dosages (1.1 to 4.0 mg/kg, daily) suggest no difference in the degree of hepatocellular vacuolation or chemical composition of the cytosol in vacuolated hepatocytes within the glucocorticoid high-dose range.

In the dogs used in our study, hematologic changes were minimal. In fact, fewer hematologic changes were identified in these dogs than were reported in dogs given 1.1 mg of prednisolone/kg, PO, daily for 35 days.[31] Modest changes in liver enzyme activity were detected, compared with findings of studies[26,29,31,33] in which dogs were given high doses of glucocorticoids parenterally. Increased protein synthesis (enzyme induction) and bile acid-facilitated enzyme elution from cell membranes are believed to contribute to increases in serum ALP and GGT activities in cholestatic disorders.[50–55] However, increased serum ALP activity in dogs with vacuolar hepatopathy is believed to derive primarily from glucocorticoid-signaled protein synthesis.[33,55] The isoenzyme initially induced after glucocorticoid exposure in dogs is the liver isoenzyme, and thereafter, the glucocorticoid-induced ALP isoenzyme progressively contributes to the total ALP activity.[33] Because induction of ALP by administration of glucocorticoids has been an inconsistent finding in studies involving different dosages of glucocorticoids and routes of administration, the modest changes in liver enzyme activity identified in the present study are not unusual. However, our findings have indicated that SAMe does not blunt glucocorticoid induction of serum ALP or glucocorticoid-induced ALP-isoenzyme activities.

Induction of haptoglobin (an acute-phase protein produced in the liver) is exquisitely responsive to glucocorticoids as well as cytokines.[56,57] In the study of this report, significantly increased serum haptoglobin concentrations were detected in each group of dogs at all sampling time points after day 0. This finding is consistent with prior observations of dogs treated with glucocorticoids and proves that the prednisolone was absorbed after administration and signaled protein synthesis and that the administration of SAMe did not blunt this effect.

In the study dogs, baseline tissue-protein concentration approximated 12.6 ± 0.2% of liver wet weight and increased to 13.8 ± 0.1% and 13.6 ± 0.1% after SAMe-prednisolone and placebo-prednisolone treatments, respectively. The reason why these findings differ from those of another study[27] in dogs with glucocorticoid-induced vacuolar hepatopathy in which hepatic protein content per liver wet weight declined 0.9-fold remains unexplained. However, our data are consistent with the known ability of glucocorticoids to selectively induce certain genes, some of which increase hepatic protein synthesis.[58–60]

Also, baseline hepatic glycogen concentration was 2.1 ± 0.1% of liver wet weight in the dogs of our study, which is a value slightly lower than that previously reported[27] for clinically normal dogs by use of a similar

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glycogen assay (3.9 ± 1.5%). Nutritional intake and duration of food withholding prior to tissue collection could have influenced the differences in results between these investigations. On induction of hepatocellular vacuolation, glycogen content increased significantly in dogs receiving SAMe-prednisolone or placebo-prednisolone (2.1- and 2.0-fold increases, compared with day-0 values, respectively) and was similar to the 2.1-fold increase from baseline reported in dogs receiving 1.2 mg of prednisone/kg, PO, daily for 15 days.[27] We had anticipated that SAMe administration would not curtail glycogen accumulation because it preserved hepatic glycogen in rats with bile duct occlusion and rats with cirrhosis induced by carbon tetrachloride.[61,62] This influence was suggested to be mediated in part by induction of glycogen synthase, the enzyme mediating hepatic glycogen synthesis. The influence of thiol-disulfide status on enzyme activity has been well studied for enzymes involved in glucose metabolism.[63] Because oxidation of cysteine groups enhances glycogen catabolism and inhibits glycogen synthesis, increased hepatic TGSH concentrations would favor glycogen accretion.[63] In our study, histologic findings in liver specimens were consistent with those of other studies in which high doses of prednisolone were given.[25–27,32] Administration of SAMe to dogs within the present study did not attenuate hepatocyte vacuolation or alter the zonal distribution of vacuolar change. However, the areas that were most consistently vacuolated (zones 1 and 2) corresponded with localization of gluconeogenic enzymes rather than with areas of glycogen synthesis (zone 3).[64]

Normally, GSH is present in high concentration in whole blood and > 98% of it is contained within the RBCs.[65,66] High TGSH concentration in erythrocytes has importance because of the close association between RBC membranes (containing polyunsaturated fatty acids) and hemoglobin (containing vulnerable sulfhydryl bonds and the transition metal iron) with high concentrations of oxygen.[67] Erythrocytes have the capacity to synthesize large amounts of GSH from amino acid precursors, interconvert RGSH and GSSG (GSH redox cycle), and extrude GSSG from the cell. Thus, their GSH homeostasis is maintained by an active and dynamic balance among biosynthesis, uptake, oxidation, recycling, and exportation. Erythrocytes function as a transit vehicle for GSH (as GSH substrates) to other tissues and provide antioxidant mediation in the systemic circulation. Intraerythrocytic GSH catabolism allows release of glycine, cysteine, and γ-carboxyglutamic acid; extracellular catabolism of GSH may occur after carrier-facilitated exportation of GSSG from erythrocytes. The findings of the present study suggest that prednisolone has a diminishing influence on GSH concentrations in RBCs. Baseline erythrocyte TGSH concentration was within the range reported for normal dogs by other investigators.[68–70] Because the blood collection technique, means of transport, assay methods, and standard controls used at each test point during the study were consistent, methodological differences cannot explain our findings. All dogs in the present study had a similar phlebotomy-related reduction in PCV (a significant difference from day-0 values was detected as early as day 14); therefore, comparable populations of young RBCs probably existed in dogs of each treatment group. Considering that some studies[65,71–73] have revealed that young erythrocytes contain higher concentrations of TGSH and a greater ability to synthesize GSH from precursor substrates than mature or aged cells, it is possible that altered RBC maturity influenced blood TGSH concentrations. Thus, it is possible that prednisolone may have diminished erythrocyte TGSH content more extensively than indicated by our measurements. However, because we did not attempt to label RBC populations and did not enumerate reticulocytes as evidence of a shifting RBC cell population, this remains speculative.[65,71,72] Although there is little information regarding the influence of glucocorticoids on erythrocyte TGSH concentrations, a similar suppressive effect has been reported in humans with mild asthma that was stabilized by administration of inhaled corticosteroids and in rats treated with high doses of dexamethasone.[74,75] In the present study, administration of prednisolone without SAMe decreased the median erythrocyte TGSH on day 42 by approximately 15%, compared with baseline values. Comparative reduction in erythrocyte GSH concentration in humans with mild asthma, which was stabilized by administration of inhaled beclomethasone dipropionate, was 6% and in young and old rats treated parenterally with dexamethasone was 33% and 36%, respectively.[74,75] It is possible that glucocorticoids may influence erythrocyte TGSH concentration through induction of membrane proteins mediating extracellular transport of GSH-*S* conjugates.[76–80] For example, induction of the **multidrug-resistance protein (MRP)** superfamily, known to facilitate the active transport of a wide variety of compounds across biological membranes, could promote extrusion of GSH from erythrocytes.[76,78–80] Furthermore, GSSG (functioning as a substrate and modulator of MRP-1) is known to exist in the plasma membranes of erythrocytes of some species (eg, humans), and it is proven that different MRPs undergo variable induction by steroid hormones.[78–80] In fact, a GSH cotransport mechanism involved with cell detoxication is proposed to influence MRP-mediated resistance to certain chemotherapeutic agents, explaining the phenomenon whereby prednisolone exposure may reduce tumor chemosensitivity to certain anticancer agents.[76] Although the data were not significantly different between treatments in our study, the erythrocyte TGSH concentration was better conserved and the erythrocyte GSSG:TSGH ratio was lower in association with SAMe administration, which might suggest a positive influence on RBC redox status or protection against oxidative injury induced by prednisolone. These effects, considered along with the significant increase in hepatic TGSH concentrations, suggest a continuum of systemic effects (perhaps derived from the liver) that may have influenced erythrocytes.

Glucocorticoid induction of hepatocyte MRP transport has been determined in vitro, and it is known that the inducible MRP-1 and MRP-2 pumps export both GSH and GSSG from liver cells.[76–78] Loss of RGSH from liver cells would directly compromise their antioxidant protection, whereas GSSG exportation

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from hepatocytes would compromise cell GSH recycling and conservation, which are normally maintained by the GSH redox cycle (ie, reduction of GSSG back to GSH). Because glucocorticoids can influence a number of enzymes that embellish or attenuate oxidant stress, GSH synthesis, and cell extrusion of GSH and GSSG, a variety of drugs in this category have the potential to impose complex effects on hepatocellular GSH and redox status. Imposed effects may vary with extenuating circumstances, such as those encountered with naturally occurring disease processes. Examples of enzymes enhanced by glucocorticoids that may variably influence hepatocellular GSH concentrations include GSH-S-transferase (which catalyzes GSH conjugation with electrophile toxicants), superoxide dismutase (which dismutates superoxide radical to hydrogen peroxide), GSH peroxidase (which detoxifies hydrogen peroxide to water in the presence of GSH), GGT (which facilitates cleavage of the γ-glutamylcysteine bond of extracellular GSH), and γ-glutamylcysteine synthetase (which is the rate-limiting enzyme for GSH synthesis).[79,81-86] Glucocorticoids also induce SAMe synthetase (the enzyme that catalyzes formation of SAMe from methionine) by influencing gene expression at a posttranscriptional level.[87] Consequently, an overall consistent influence of glucocorticoids on hepatic GSH concentrations has not been identified in various animal models of disease, at least in the small number of relevant investigations published to date.

In the study of this report, it was not surprising that treatment with SAMe significantly increased hepatic TGSH and RGSH concentrations from baseline values. Similar effects have been detected in human patients with liver disease, in animals used in models of liver disease, and through in vitro work. However, a synergistic influence derived from coadministration of SAMe and prednisolone may have contributed to our findings. The different response of erythrocytes and hepatocytes in the present study could reflect the comparatively limited ability of RBCs to increase GSH synthesis or their comparatively enhanced extrusion of GSH conjugates or GSSG. Although there was no significant difference in liver TGSH concentration between treatments, the significant increase from baseline of both the absolute and relative (percentage) change of hepatic GSH concentration, along with the greater hepatic RGSH:TGSH ratio achieved with SAMe treatment, suggests that SAMe administered via the oral route exerts a biologic influence on the liver. Our findings also may reflect an ability of SAMe to attenuate oxidative influences imposed by glucocorticoids. The finding of another study[16] that indicated that dogs with severe glucocorticoid-induced vacuolar hepatopathy may develop subnormal hepatic TGSH concentrations concurs with this consideration.

Hepatocytes in dogs treated long-term with high doses of prednisolone develop subcellular biochemical changes consistent with increased organelle fragility.[29] This includes lysosomal fragility, similar to effects of high doses of prednisolone in neural tissues.[29,88] Lysosomal integrity is important because the release of lysosomal hydrolases can damage cell and organelle membranes. The suggestion that glucocorticoids may impair hepatocellular resistance to certain forms of injury or may enhance tissue injury is not novel. Serum biochemical and histologic findings in dogs with hypotensive hepatic injury, followed by high-dose glucocorticoid exposure (ie, dexamethasone administered at a dosage of 2.2 mg/kg, SC, q 12 h), substantiated that such treatment compromised tissue defense and recovery.[32] Although the mechanism of this influence is complex and not fully explained, findings in the present study and other work in dogs with severe glucocorticoid vacuolar hepatopathy associated with impaired liver function (ie, reduced organic anion clearance) indicate that high-dose glucocorticoid treatment compromises hepatocytes in complex ways, which may include imposition of oxidant stress.

Clinical and investigative studies[11–16] have substantiated an important role of membrane oxidation as a central pathomechanism of most forms of liver disease. Mechanisms resulting in membrane damage are numerous and complex, involving accumulation of membranocytolytic bile acids, direct and indirect hepatotoxins, toxic adducts, transition metals (eg, iron and copper), inflammatory mediators, and cytokines and adverse effects imposed by activated Kupffer and inflammatory cells and infectious organisms.[89] Many chronic necroinflammatory and cholestatic liver disorders in dogs are treated with glucocorticoids at dosages comparable to those used in the study of this report. Considering our previous demonstration of decreased hepatic TGSH concentrations in dogs with necroinflammatory and cholestatic liver disease, use of SAMe as a therapeutic maneuver to increase hepatocellular GSH concentrations and improve tissue redox status seems reasonable.[16] Maintaining an optimal RGSH:TGSH ratio in hepatocytes is important for their normal metabolism and homeostasis because thiol status regulates a myriad of essential cell functions (ie, gene expression, cell signaling, and mediation of inflammation and apoptosis).[90]

Findings of the present study have verified that oral administration of an enteric-coated tablet containing the 1,4-butanedisulfonate salt of SAMe[a] (20 mg/kg), given after withholding of food, delivers bioavailable SAMe that can influence circulating and tissue antioxidant status in dogs. Results have suggested that glucocorticoids may impose oxidant stress and that SAMe may attenuate this influence. Although SAMe administration did not prohibit development of the classic features of glucocorticoid hepatopathy in dogs, it did significantly increase liver TGSH concentration and appeared to favorably influence RBC and hepatic redox status. Although the findings of the present study were not complicated by the oxidative challenges imposed by necroinflammatory and cholestatic liver disease, the effects of SAMe in the dogs of this report would also be expected to yield benefits in dogs with those diseases.[16,89,90] Considering that many of the necroinflammatory and cholestatic liver disorders affecting dogs are treated with glucocorticoids at high dosages, application of SAMe in this context may be appropriate.

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a. Denosyl SD4, Nutramax Laboratories Inc, Edgewood, Md.
b. Lithium heparin vacutainers, Becton-Dickinson, Franklin Lakes, NJ.
c. EDTA vacutainers, Becton-Dickinson, Franklin Lakes, NJ.
d. S+IV automated cell-counting instrument, Coulter Electronics, Hialeah, Fla.

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e. Hitachi 917 automated analyzer, Roche Diagnostics, Indianapolis, Ind.
f. Sigma Bile acid reagent test kit, Sigma Diagnostics, St Louis, Mo.
g. Indocyanine green (Cardiogreen), freeze-dried, Sigma Chemical Co, St Louis, Mo.
h. Reduced glutathione, 98% pure, Sigma Chemical Co, St Louis, Mo.
i. Oxidized glutathione, 98% pure, Sigma Chemical Co, St Louis, Mo.
j. Butylated hydroxy toluene, Sigma Chemical Co, St Louis, Mo.
k. 1,1,3,3-tetraethoxypropane (97% pure), Sigma Chemical Co, St Louis, Mo.
l. SPE extraction cartridge, Oasis HBL, Waters Chromatography, Milford, Mass.
m. Biophase ODS, Bioanalytical Systems Inc, West Lafayette, Ind.
n. Waters radial compression guard column unit (Guard-Pak equipped with a µBondapak insert), Waters Corp, Milford, Mass.
o. 2-vinylpyridine, 97%, Aldrich Chemical Co, Milwaukee, Wis.
p. Bovine serum albumin, 35% solution, Sigma Chemical Co, St Louis, Mo.
q. Hoecsht 33258, Aldrich Chemical Co, Milwaukee, Wis.
r. DNA, sodium salt type 1, highly polymerized from calf thymus, desiccated, Sigma Chemical Co, St Louis, Mo.
s. Proprietary information, investigators brochure, ademetionine-SD4, Research and Development, Knoll AG, Regulatory Affairs Department, BioResarch Spa, BASF Pharma, Milano, Italy.

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