Journal of Feline Medicine and Surgery (2003) **5**, 69–75
doi:10.1016/S1098-612X(02)00017-7

jfms

S-adenosylmethionine (SAMe) in a feline acetaminophen model of oxidative injury

CB Webb[1*], DC Twedt[1], MJ Fettman[2], G Mason[2]

[1] *Department of Clinical Sciences and* [2] *Department of Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, CO 80523, USA*

S-adenosylmethionine (SAMe) is reported to have hepatoprotective and antioxidant functions. Acetaminophen (paracetamol) was used to induce oxidative damage in cats, and to then determine the effect of SAMe treatment on erythrocyte morphology, PCV, liver histopathology, thiobarbituate reacting substances (TBARS), reduced glutathione (GSH), and oxidised glutathione (GSSG).

Cats receiving acetaminophen had a significant increase in methemoglobin and Heinz body production. A significant effect for the interaction of time and treatment was found for Heinz body production and changes in PCV. No significant changes were found in blood or hepatic TBARS. Blood GSH increased significantly in all cats, while the blood GSH:GSSG ratio tended to increase the most in cats given acetaminophen only. The hepatic GSH:GSSG ratio tended to increase in cats given SAMe and decrease in cats given acetaminophen, but this effect was not significant.

SAMe protected erythrocytes from oxidative damage by limiting Heinz body formation and erythrocyte destruction and maybe useful in treating acetaminophen toxicity.

Date accepted: 14 February 2002

© 2003 ESFM and AAFP. Published by Elsevier Science Ltd. All rights reserved.

S-adenosylmethionine (SAMe) has been utilised in the management of a variety of human diseases but there are a limited number of controlled studies on the use of SAMe therapy in small animals (Center et al 1999, 2000). SAMe is reported to have both hepato-protective and antioxidant properties, and is involved in a number of metabolic pathways designed to preserve cell membrane function and protect cellular constituents from oxidative damage (Williams & Lieber 1990). Preliminary studies suggest that SAMe supplementation increases hepatic glutathione concentrations in normal cats (Center et al 2000), and prevents glutathione depletion in dogs with steroid-induced hepatopathy (Center et al 1999).

Cats have limited ability for acetaminophen (paracetamol) metabolism (Welch et al 1966). The majority of acetaminophen metabolism occurs through the cytochrome P450 system, from which highly reactive free-radical molecules are produced (Savides et al 1984). Reduced glutathione is the primary intracellular free radical scavenger responsible for sequestering the reactive acetaminophen metabolites (Fettman 1991). Following oxidative damage, the ratio of reduced glutathione (GSH) to oxidised glutathione (GSSG) declines if GSH stores become depleted (Center & Warner 2000). Evidence of erythrocyte oxidative damage can be detected by the formation of methemoglobin and Heinz bodies, and thiobarbituate reacting substances (TBARS), an end product of membrane peroxidation.

The purpose of this study was to investigate the effect of SAMe administration on markers of haematologic and hepatocellular oxidative stress following acetaminophen administration in the cat.

Materials and methods

Cats

Eighteen healthy domestic shorthaired cats were used for this study. The mean age was 2.5 years

*Correspondence to: Craig B. Webb, Colorado State University, 300 West Drake Road, Fort Collins, CO 80523, USA. E-mail: cbwebb@colostate.edu

1098-612X/03/020069+07 $30.00/0 © 2003 ESFM and AAFP. Published by Elsevier Science Ltd. All rights reserved.

(range 2.0–2.7), and the mean weight was 4.2 kg (range 3.1–5.6). Cats were randomly assigned to one of three groups of six animals. Group one was treated with SAMe[a] (SAMe), a second group received acetaminophen[b] (ACE), and the third group received acetaminophen followed by SAMe treatment (ACE-SAMe). AU procedures were performed in accordance with Colorado State University Animal Care and Use Committee Guidelines.

Initial data collection

An initial biochemical profile and haemogram were performed on all cats. Liver samples were obtained using standard laparoscopic biopsy techniques. Cats were sedated with acepromazine (0.25 mg/cat) and oxymorphone (0.06 mg/kg), titrated to effect with ketamine, and received a local carbocaine anaesthetic at sites of trochar placement. Three liver samples (approximately 32 mg each) were obtained. Samples were stored in formalin for histopathology or immediately snap frozen in liquid nitrogen and stored in a –70°C freezer for analysis of GSH, GSSG, TBARS, and protein. Following initial liver sample collection there was a 4-week recovery period prior to further intervention.

Experimental design

The experimental timetable was the same for all animals, and all medications were given orally. Following the initial 4-week recovery period the treatment protocol was begun and that time was designated as Day 0. On Day 0 blood was collected for PCV, total protein, methemoglobin, GSH, GSSG, TBARS, and percent Heinz bodies, and treatments were then initiated. The SAMe group received 180 mg of SAMe twice daily for a total of 3 days, then 90 mg SAMe twice daily for the remainder of the 14-day study period. The ACE group received 90 mg/kg acetaminophen once, and no further medications throughout the 14-day study period. The ACE-SAMe group received 90 mg/kg acetaminophen once, and 1 h later SAMe treatment was started using the SAMe only group protocol.

Four hours following the initial treatment, methemoglobin was determined. On Day 3, PCV, GSH, GSSG, ALP, ALT, AST, TBARS, and percent of erythrocytes with Heinz bodies were determined and a second liver biopsy was obtained for GSH, GSSG, TBARS, protein content and histopathology. Blood samples were obtained on Day 7 and 14 for PCV, GSH, GSSG, TBARS, and percent Heinz bodies.

Heinz body determination and PCV

Blood films were stained with brilliant cresyl blue, and 1000 erythrocytes were counted. Erythrocytes containing Heinz bodies ≥ 1 µm in diameter were counted, and the results were expressed as a percentage of total erythrocytes. The PCV was expressed as percent of total blood volume.

Methemoglobin analysis

Methemoglobin concentration was determined in heparinised blood by measuring a change in absorbance at 630 nm, before and after addition of potassium cyanide, and expressed as a percentage of total haemoglobin (Young & Trimble 1991).

Blood TBARS

Butylated hydroxytoluene (BHT 0.09 M) was added to the blood sample to reduce enzymatic membrane oxidation during sample processing. Whole blood collected in sodium heparin was washed in cold isotonic saline, and suspended in buffer containing BHT and thiobarbituric acid (0.03 M). The spectrophotometric absorbance of the supernatant was recorded at 535 nm and compared with TBARS standards (range = 24.3 µm through 1.52 µm malondialdehyde). The result was normalised using the samples PCV, and expressed as µmol/100 ml RBC (St. Omer & McKnight 1980).

Liver TBARS

Liver tissue was weighed, homogenised in BHT, and processed as above for blood TBARS determination. Results were normalised to liver protein content.

Liver tissue protein

Liver protein concentrations were determined using the standard Lowry method (Lowry et al 1951).

[a]Denosyl® SD4, Nutramax Laboratories, Inc., enteric coated, individually sealed in foil blister packs, pure and stabilised 1, 4-butanedisulfonate salt of S-adenosylmethionine
[b]Tylenol, McNeil-PPC. Inc.

Blood glutathione

Glutathione was quantified from EDTA-preserved blood using an enzymatic assay (Tietze 1969).[c] The assay specifications demonstrate linearity to a sample concentration of 2400 µM GSH, recovery of 98%, a lower limit of detection (sensitivity) of 0.54 µM, and no interfering effects with tested analogues (specificity).[d] For GSSG, a thiol-scavenging reagent, 1-Methyl-2-vinyl-pyridium trifluoromethane sulfonate (M2VP) is added to rapidly scavenge GSH and eliminate continued oxidation of GSH to GSSG after sample acquisition. Glutathione reductase, NADPH, and the chromogen 5,5′-Dithiobis-(2-nitrobenzoic acid) (DTNB), were added sequentially to the sample, and the change in absorbance at 412 nm was measured spectrophotometrically. Total (GSH + GSSG) glutathione was quantified in EDTA-preserved blood without the addition of M2VP. Measured levels of reduced (GSH) and oxidised (GSSG) glutathione in the blood were normalised using the corresponding PCV, and recorded as µmole/100 ml RBC.

Liver glutathione

Liver GSH and GSSG were quantified using the same enzymatic assay[c] described above for blood. Liver tissue was weighed, and homogenised in phosphoric acid with, or without M2VP. The volume of phosphoric acid was adjusted to maintain a constant final homogenate volume of 400 µl. Results were normalised to liver protein content.

Liver histopathology

Liver tissue was processed in a standard manner and evaluated histologically for morphologic changes by a single pathologist (GM) blinded to the day and groupings.

Statistical analysis

The Kolmogorov-Smirnov test was applied to all data using a computer statistical software package (StatView®) in order to determine normality of distribution for further parametric analysis. Analysis of variance for repeated measures was used to evaluate parameters for the effect of treatment, time, and the time-treatment interaction. Testing for significant differences between groups for variables at individual time points was done post hoc by applying the Fishers LSD test to the analysis of variance. Significance was assumed at $P \leq 0.05$ for all comparisons.

[c]BIOXYTECH® GSH/GSSG-412™ assay

[d]Bioxytech® GSH/GSSG-412™ Catalog #21040, 'Colorimetric determination of reduced and oxidised glutathione'. Kit instructions.

Results

Histopathology of liver tissue prior to any treatments did not reveal significant lesions in any of the cats. There were no significant differences between groups for pre-treatment PCV and total protein, or liver enzyme activities ($P > 0.05$). None of the cats had ALP or ALT enzyme activities outside the normal reference range prior to treatment. Liver enzyme activities remained within the reference range for all of the SAMe control cats. Two of the cats that received acetaminophen (one from the ACE group and one from the ACE-SAMe group) died or were euthanised within 36 h after receiving the drug. Both animals were administered acetaminopen on the same morning, and monitored multiple times in individual cages during the subsequent 16 h. Both cats displayed similar symptoms observed in other cats given acetaminophen including moderate lethargy and discoloration of mucous membranes. Both animals ate within that 16-h time period and were returned to group housing. The ACE treated cat was found dead the following morning, approximately 24 h after acetaminophen administration. Unfortunately, that cat was disposed of prior to obtaining a necropsy, despite an established protocol for post-mortem investigation. The second ACE-SAMe treated cat was discovered to be in distress 36 h following initial treatment. This cat failed to respond to supportive care, and was subsequently euthanised. Liver histology from the second cat showed severe centrilobular hydropic degeneration with necrosis. Serum chemistry collected prior to euthanasia revealed marked increases in liver enzyme activities (ALT >250 times normal, ALP 1.5 times normal).

Subsequent to these deaths, additional post-treatment monitoring was instituted for cats receiving acetaminophen, but no other significant adverse reactions were ever observed in the remaining cats. One cat in the ACE group had increased ALT concentration on Day 3 (ALT >4.4 times normal) and two cats from the ACE-SAMe group had increased ALT activity on Day 3 (>10 times normal and >24 times normal respectively).

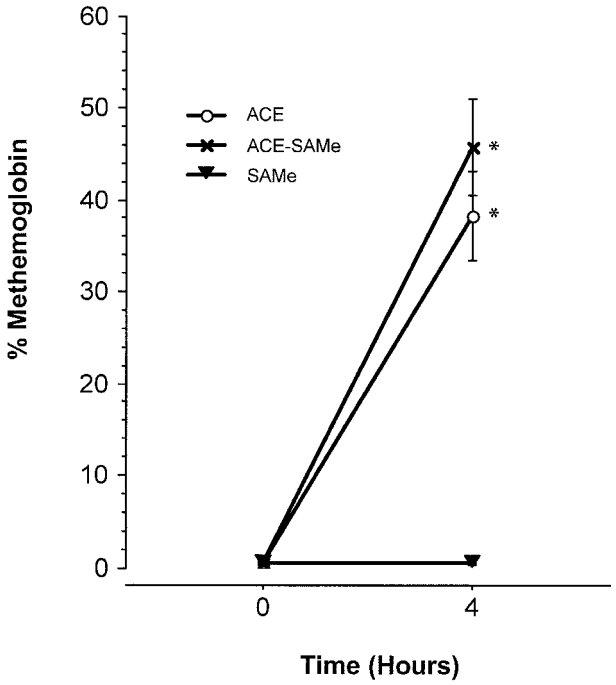

Fig 1. Change in percent methemoglobin (mean±standard error) over time. *Represents a significant difference from the SAMe control group ($P<0.0001$).

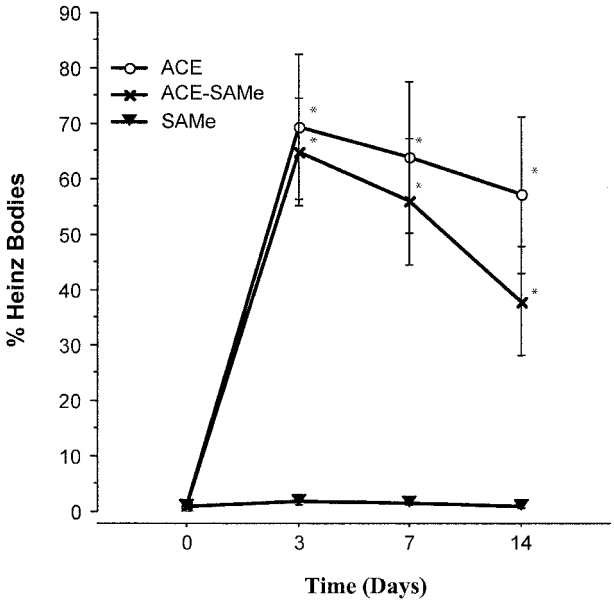

Fig 2. Change in percent Heinz bodies (mean±standard error) over time. *Represents a value significantly different from the SAMe control group ($P<0.001$).

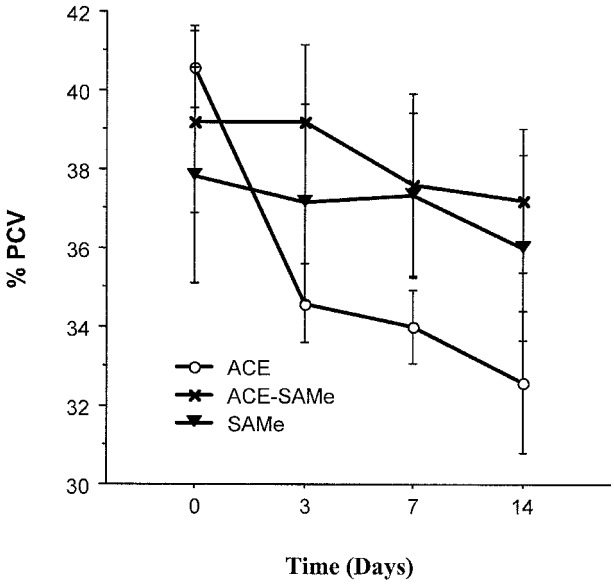

Fig 3. Change in percent PCV (mean±standard error) with time. The interaction between treatment and time among groups is significant ($P = 0.008$).

All eighteen animals had methemoglobin concentrations less than 1.0% prior to receiving treatment. There was no increase in methemoglobin in the SAMe group 4 h following treatment. There was a significant interaction of time and treatment for methemoglobin formation ($P<0.001$). Cats that received acetaminophen, or acetaminophen plus SAMe had a significant ($P<0.0001$) increase in methemoglobin (mean = 42%, range 21–56.7) compared to the SAMe group. There was no significant difference in the magnitude of the methemoglobin increase between the ACE and the ACE-SAMe group (Fig 1).

The percent Heinz bodies was less than 1.0% for all cats on Day 0. There was a significant interaction of time and treatment for Heinz body formation ($P<0.001$). The percent Heinz bodies were significantly ($P<0.0001$) increased in both the ACE group and the ACE-SAMe group by Day 3 (Fig 2), however the values were not significantly different between the ACE and the ACE-SAMe group for any single time point ($P > 0.05$).

A significant ($P = 0.008$) effect for the interaction of time and treatment was found for PCV among the three groups (Fig 3), although values at any single time point were not significantly different ($P > 0.05$). The mean PCV of cats in the ACE group decreased 20% from Day 0 to Day 14, the PCV of cats in the SAMe group decreased 6.0%, and the PCV of cats in the ACE-SAMe group decreased 5.0%.

There was no significant interaction of time and treatment for TBARS formation in either the liver or blood. There was a significant ($P = 0.0027$) effect for the interaction of time and treatment on the blood GSH:GSSG ratio among groups (Fig 4). Those cats receiving only acetaminophen tended to have the greatest increase in blood GSH:GSSG

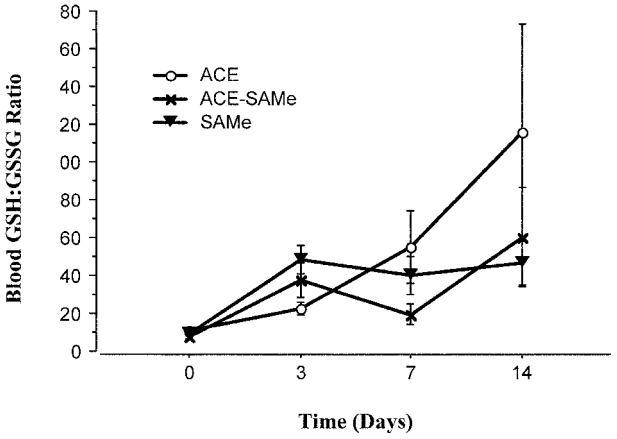

Fig 4. The ratio of reduced (GSH) to oxidised (GSSG) glutathione in blood (mean±standard error). The interaction between and time among groups is significant ($P = 0.0027$).

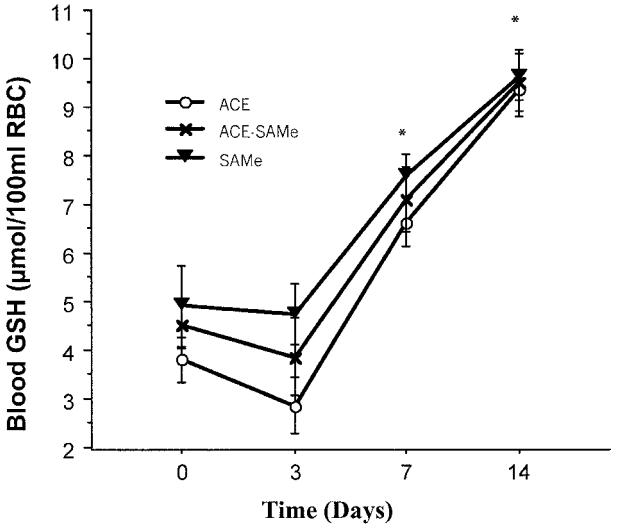

Fig 5. Change in blood GSH with time (mean±standard error). *Significant difference from Day 0 ($P<0.0001$).

ratio, but there were no significant differences between groups at individual time points. The concentration of blood GSH increased significantly ($P<0.0001$) for all three groups (Fig 5) while blood GSSG concentration did not change significantly in any group ($P > 0.05$).

There was no significant interaction of time and treatment for the GSH:GSSG ratio in liver tissue ($P > 0.05$), although this ratio tended to increase in cats given SAMe and decrease in cats given acetaminophen (Fig 6). The interaction of time and treatment for hepatic GSH and GSSG was also not significant ($P > 0.05$).

No significant changes were seen in liver histopathology between Day 0 and Day 3 in any of the

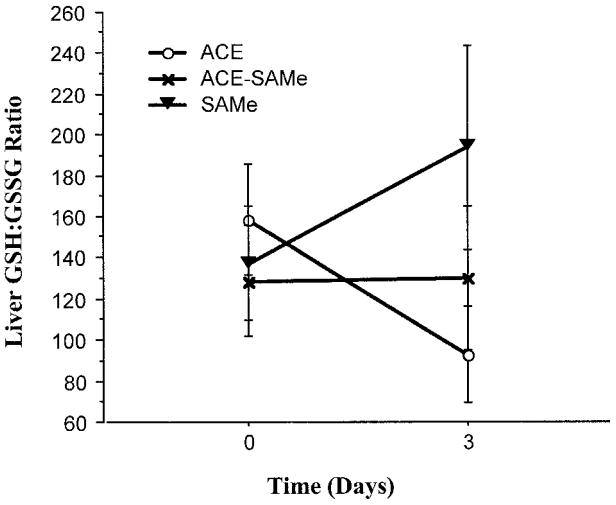

Fig 6. Change in the ratio of reduced glutathione (GSH) to oxidised glutathione (GSSG) in the liver over time (mean±standard error).

cats, with the exception of those changes already described for the cat that died in the ACE group.

Discussion

Acetaminophen has been shown to cause haematologic oxidative damage in cats (Finco et al 1975). The purpose of this study was to investigate the effect of SAMe administration on markers of haematologic and hepatocellular oxidative stress following acetaminophen administration in the cat.

The dose of acetaminophen (90 mg/kg) for this study was chosen to induce measurable oxidative damage with minimal adverse side effects based on a report of 45 cats given this dose with no reported mortality and minimal morbidity (Allison et al 2000). This dose is also significantly lower than the lowest fatal dose (143 mg/kg) found in a review of the literature (Gaunt et al 1981), and one prior report of six cats receiving 120 mg/kg acetaminophen reported no mortality (Savides et al 1984). Despite this, two of the animals in this study died at 24 and 36 h following acetaminophen ingestion. Three other cats showed a significant elevation in ALT activity on Day 3 following acetaminophen administration, while ALT activity remained normal in the remaining seven cats given the drug. These results would tend to suggest a significant degree of individual variation in the metabolism of acetaminophen and processing of oxidative stress between cats.

Acetaminophen treated cats showed a rapid increase in methemoglobin formation within 4 h.

The absence of a significant difference in methemoglobin levels between the ACE and ACE-SAMe groups suggests that SAMe administration had no effect on the degree of methemoglobin production 4 h following acetaminophen administration. This is consistent with a previous report in which acetylcysteine treatment had no effect on methemoglobin levels in acetaminophen treated cats 4 h following administration (St. Omer & McKnight 1980).

Because the feline spleen is inefficient at removing Heinz bodies from the blood, they serve as persistent markers of oxidative damage (Christopher et al 1990). Heinz bodies were found in both the ACE and the ACE-SAMe groups on Day 3. Rapid oxidative damage may have taken place before SAMe could have a significant effect on initial Heinz body formation. There was a significant effect of time and treatment for Heinz body formation, with the ACE-SAMe treated group tending to have a more rapid decline in Heinz body numbers.

There was a significant effect of time and treatment for PCV. The greatest fall in PCV was observed in the ACE only group; by Day 14 the decline in PCV for the ACE group was 15% greater than the decline in PCV for SAMe treated cats. The mild drop in PCV for the SAMe control group is likely the result of blood loss following multiple liver biopsies, acquisition of multiple blood samples, or dilution secondary to fluids received during the laparoscopy procedures. Changes in red blood cell membrane fragility may explain the significant time treatment interaction on the PCV, which was most evident at Day 14. A significant decrease in erythrocyte osmotic fragility has been previously demonstrated in normal cats treated with SAMe (Center et al 2000). S-adenosylmethionine appeared, with time, to have a protective effect on PCV in acetaminophen treated cats, although the values were not significantly different at any single time point. This finding is consistent with previous reports of *N*-acetylcysteine therapy preserving the PCV in acetaminophen treated cats (Gaunt et al 1981).

The peroxidation of membrane lipids is a common sequelae of cellular oxidative damage (Fettman et al 1999). One quantifiable end product of lipid peroxidation is TBARS. It was expected that the concentration of blood TBARS would increase in the ACE group and possibly the ACE-SAMe group. However, no significant changes ($P > 0.05$) or trends were observed in blood TBARS concentrations. The reason TBARS failed to show significant oxidative damage in this model may be that excessive non-lipid components dominated the assay (Lapenna et al 2001) or perhaps the level of oxidative damage produced by the dose of acetaminophen did not cause measurable erythrocyte membrane peroxidation.

Further evidence of oxidative damage can be determined by changes in the ratio of reduced (GSH) to oxidised (GSSG) glutathione in the blood. A reduction in the ratio indicates an oxidative state resulting in utilisation of reduced GSH with subsequent formation of oxidised GSSG (Freeman & Crapo 1982). In this study the opposite was found, with the blood GSH:GSSG ratio being the greatest for the acetaminophen control group by Day 14. The major factor influencing the GSH:GSSG ratio is the absolute concentration of reduced glutathione. The increase in blood GSH levels in the SAMe cats is similar to an increase in blood GSH previously reported for acetaminophen intoxicated cats treated with *N*-acetylcysteine (Gaunt et al 1981), normal cats supplemented with cysteine (Fettman et al 1999), and normal cats treated long term with SAMe (Center et al 2000). The increase in blood GSH concentration and GSH:GSSG ratio in the ACE group was unexpected. These findings raise the possibility that some independent factor is impacting blood glutathione activity in this particular model, or that blood GSH concentration was depleted shortly after acetaminophen administration, but had rebounded to greater than normal levels by Day 3.

Reduced glutathione (GSH) serves as the major soluble free radical scavenger in the liver, preventing oxidative damage and lipid membrane peroxidation. As in the blood, a reduction in the ratio of hepatic GSH:GSSG should be indicative of oxidative damage (Freeman and Crapo 1982). Changes in the hepatic GSH:GSSG ratio were not significantly different between groups, but the tendency for the GSH:GSSG ratio to increase in the SAMe control group, decrease in the acetaminophen only group, and remain stable in the ACE-SAMe group, are consistent with a SAMe-mediated protection of hepatic glutathione reducing power. Hepatocellular glutathione levels may have been sufficient to prevent significant oxidative damage without depletion of GSH, or hepatic GSH may have been replenished through the reduction of GSSG prior to Day 3. Measuring hepatic glutathione levels earlier than Day 3 may have uncovered significant evidence of oxidative damage.

In summary, acetaminophen caused significant oxidative damage in the erythrocytes of cats. S-adenosylmethionine therapy, instituted 1 hour following drug administration, showed evidence of protecting against acetaminophen-induced changes in the formation of Heinz bodies and the decline in PCV over time. Pre-treatment with SAMe may have further prevented the oxidative damage observed in the ACE-SAMe group, as was seen in a feline model using pre-treatment with a bioflavonoid followed by the same dose of acetaminophen (Allison et al 2000). However, the current study was designed, in part, to mimic a clinical setting where SAMe administration would likely occur after acetaminophen intoxication. Nevertheless, SAMe appeared to have a protective effect for some parameters in cats given acetaminophen, and further work is needed to decide if SAMe can also be a useful agent as an adjunct to traditional treatments for cats experiencing diseases involving oxidative stress.

Acknowledgements

The study was funded by the Nutramax Laboratories, Inc. Special thanks to Kris Obssuth, Mark Mathes, JoAnn Harper, and Kelly Avila for their technical assistance.

References

Allison RW, Lassen D, Burkhard MJ et al. (2000) Effect of a bioflavonoid dietary supplement on acetaminophen-induced oxidative injury to feline erythrocytes. *Journal of American Veterinary Medical Association* **217**, 1157–1161.

Center SA, Randolph JF, Warner K et al. (2000) Influence of SAMe on erythrocytes and liver tissue in healthy cats, (abstract). *Journal of Veterinary Internal Medicine* **14**, 357.

Center SA, Warner K (2000) Hepatic glutathione concentrations in dogs and cats with spontaneous liver disease, (abstract). *Journal of Veterinary Internal Medicine* **14**, 318.

Center SA, Warner K, Hofmann WE et al. (1999) Influence of S-adenosylmethionine (SAMe) on metabolic and morphologic hepatocellular features induced by chronic glucocorticoid administration in dogs, (abstract). *Journal of Veterinary Internal Medicine* **13**, 253.

Christopher MM, White JG, Eaton JW (1990) Erythrocyte pathology and mechanisms of Heinz body-mediated hemolysis in cats. *Veterinary Pathology* **27**, 299–310.

Fettman MJ (1991) Comparative aspects of glutathione metabolism affecting individual susceptibility to oxidant injury. *The Compendium of Continuing Education* **13**, 1079–1091.

Fettman MJ, Valerius KD, Ogilvie GK et al. (1999) Effects of dietary cysteine on blood sulfur amino acid, glutathione, and malondialdehyde concentrations in cats. *American Journal of Veterinary Research* **60**, 328–333.

Finco DR, Duncan JR, Schall WD (1975) Acetaminophen toxicosis in the cat. *Journal of American Veterinary Medical Association* **166**, 469–472.

Freeman BA, Crapo JD (1982) Biology of disease: Free radicals and tissue injury. *Laboratory Investigation; A Journal of Technical Methods and Pathology* **47**, 412–426.

Gaunt SD, Baker DC, Green RA (1981) Clinicopathologic evaluation of N-acetylcysteine therapy in acetaminophen toxicosis in the cat. *American Journal of Veterinary Research* **42**, 1982–1984.

Lapenna D, Ciofani G et al. (2001) Reaction conditions affecting the relationship between thiobarbituric acid reactivity and lipid peroxides in human plasma. *Free Radical Biology and Medicine* **31**, 331–335.

Lowry OH, Rosebrough NJ, Farr AL et al. (1951) Protein measurement with the Folin Phenol Reagent. *Journal of Biological Chemistry* **193**, 265–275.

St. Omer VV, McKnight ED (1980) Acetylcysteine for treatment of acetaminophen toxicosis in the cat. *Journal of American Veterinary Medical Association* **176**, 911–913.

Savides MC, Oehme FW, Nash SL et al. (1984) The toxicity and biotransformation of single doses of acetaminophen in dogs and cats. *Toxicology and Applied Pharmacology* **74**, 26–34.

Tietze F (1969) Enzymatic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Analytical Chemistry* **27**, 502–520.

Welch RM, Conney AH, Burns JJ (1966) The metabolism of acetophenetidin and N-acetyl-p-aminophenol in the cat. *Biochemical Pharmacology* **15**, 521–531.

Williams R, Lieber CS (1990) The role of SAMe in the treatment of liver disease. *Drugs* **40**, 1–2.

Young IS, Trimble ER (1991) Measurement of malondialdehyde in plasma by high performance liquid chromatography with fluorimetric detection. *Annals of Clinical Biochemistry* **28**, 504–508.