Therapeutic Use of Cytoprotective Agents in Canine and Feline Hepatobiliary Disease

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KEYWORDS

• S-adenosylmethionine • N-acetylcysteine • Ursodeoxycholate
• Silymarin • Vitamin E • Glutathione

Hepatocytes by virtue of their pivotal role in metabolism and their anatomic juxtaposition between the intestinal lumen and the systemic circulation are uniquely susceptible to injury. As the principal site of metabolism and detoxification of endogenous metabolites, drugs, and xenobiotics, hepatocytes are routinely exposed to potentially toxic reactive intermediates. Because hepatocytes receive the majority of their blood supply from a venous rather than an oxygen-rich arterial circulation, they are susceptible to ischemic injury. In addition, as this blood supply drains, the gastrointestinal tract hepatocytes are exposed to anything toxic that is absorbed by enterocytes, including bacterial by-products and components such as lipopolysaccharide. The liver is also home to a large population of macrophages, the Kupffer cells, which stand poised and ready for activation with subsequent release of inflammatory and toxic cytokines. These cytokines, particularly tumor necrosis factor alpha (TNF-α), can augment and perpetuate liver injury.[1] Additionally, the liver contains a population of vitamin A–storing stellate cells that can transform during liver injury (under the influence of another cytokine, transforming growth factor beta [TGF-β]) into extracellular matrix–producing myofibroblasts that lead to hepatic fibrosis.[1,2]

Given this hostile environment, hepatocytes have developed several ways to protect themselves from harm. These protections include enzymatic (catalase, superoxide dismutase [SOD], and glutathione [GSH] peroxidase and transferase) and nonenzymatic (GSH, vitamin E, ascorbate) defense mechanisms.[2,3] Hepatocytes also respond to toxic insults by initiating intracellular prosurvival signaling pathways. These pathways are controlled by hormones (eg, glucagon) and growth factors (eg,

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hepatocyte growth factor), and work through the modulation of survival kinases.[4] Many medicinal, nutraceutical, and botanic extracts have cytoprotective properties in the liver.[3–12] These agents enhance natural defense mechanisms to inhibit inflammation and fibrosis, prevent apoptosis, or protect against oxidant injury by maintenance of an appropriate redox balance.

MECHANISMS OF HEPATOCYTE CELL DEATH

Injured hepatocytes die by either apoptosis or necrosis, and both forms of cell death accompany naturally occurring hepatobiliary disease in human and veterinary patients.[13,14] Necrosis is a random, nonenergy-dependent event that occurs secondary to overwhelming cellular damage, particularly to mitochondria. Severe mitochondrial damage that results in ATP depletion leads to widespread membrane disruption, loss of plasma membrane integrity, osmotic imbalances, cell swelling, and, ultimately, cell rupture. The subsequent release of cellular contents generates an intense inflammatory response.[13,14]

Apoptosis is a genetically controlled pathway of cell death that requires maintenance of mitochondrial ATP generation. Membrane integrity is preserved, and the resultant apoptotic bodies are cleared by phagocytic cells without stimulating an inflammatory response. Apoptosis relies on the sequential activation of cellular proteases called caspases. Caspases are classified as initiator (caspases 8, 9, and 10) or effector caspases (caspase 3). Initiator caspase activation occurs by either an extrinsic death receptor or an intrinsic mitochondrial-mediated pathway. In the death receptor pathway, binding of death receptor ligands such as TNF-α or Fas to their respective death receptor causes receptor oligomerization and activation. This activation results in the recruitment and binding of adapter molecules which, in turn, recruit the initiator caspases 8, 10, or both to the death receptor. This recruitment results in cleavage and activation of caspase 8 or 10. In type II cells such as hepatocytes, caspase 8 and 10 cleave a cytosolic protein, called Bid, to generate tBid.[14] tBid leads to mitochondrial translocation or activation of the pro-apoptotic proteins Bax and Bak. Once activated, Bax and Bak lead to permeabilization of the outer mitochondrial membrane. Mitochondrial outer membrane permeabilization (MOMP) is accompanied by the formation of a pore and the release of inner mitochondrial matrix protein, cytochrome C, into the cytosol. Cytochrome C stimulates the assembly of the apoptosome, a complex of Apaf-1 (apoptotic protease activating factor 1), procaspase 9, and ATP. Caspase 9 is activated within the apoptosome and goes on to cleave and activate effector caspase 3. Once activated, caspase 3 performs the demolition phase of apoptosis and is responsible for initiating the destruction of cellular components and, ultimately, for the morphologic changes that characterize apoptosis, such as nuclear condensation and DNA fragmentation. In the intrinsic mitochondrial pathway, the trigger for apoptosis is cellular stress such as exposure to ultraviolet radiation, growth factor withdrawal, microtubular disruption, or DNA damage. These signals lead to direct Bax/Bak activation and subsequent MOMP leading to cytochrome C release.[13,14]

OXIDATIVE STRESS

Oxidative stress is defined as an imbalance between oxidant and antioxidant systems (such as an excess of reactive oxygen species [ROS] or a deficiency in antioxidants) in the cell which leads to tissue damage.[2] Oxidative stress has a major role in most forms of hepatobiliary injury.

Normally, the level of ROS in the cell is maintained by a balance between production via aerobic metabolism and elimination via antioxidant systems. In health, most ROS are generated via oxidative phosphorylation in the mitochondria. In hepatobiliary disease, activated inflammatory cells (neutrophils, Kupffer cells), cytochrome P450 enzymes (particularly uncoupled CYP2E1 isoforms), and damaged mitochondria all contribute to ROS production.[2] ROS cause cell damage via a variety of mechanisms, including the oxidation of lipids, proteins, and DNA, and the generation of toxic species (peroxides, alcohols, aldehydes, and ketones). Oxidative stress may also activate pro-apoptotic protein kinases (eg, c Jun N-terminal kinase [JNK]), proinflammatory transcription factors (nuclear factor kappa beta [NF-κβ]), and modulators of apoptosis (caspases, death receptors).[2]

Enzymatic Antioxidant Pathways

The liver possesses a complex, interactive, antioxidant network that can be divided into enzymatic and nonenzymatic pathways. Enzymatic antioxidant pathways include SOD, catalase, and GSH peroxidases. SOD, found in the cytosol and the mitochondria, catalyzes the dismutation of superoxide anion to hydrogen peroxide.[3] Catalase, located predominantly in peroxisomes, catalyzes the conversion of hydrogen peroxide to water. GSH peroxidases, a family of cytosolic and mitochondrial enzymes, convert lipid and hydrogen peroxides to water and stable alcohols by oxidizing reduced GSH to its disulfide form (GSSG) (**Fig. 1**).[3,15]

Nonenzymatic Antioxidant Pathways

Nonenzymatic defenses include GSH, vitamin E, and ascorbate. GSH is a ubiquitously expressed tripeptide of cysteine, glycine, and glutamine. It is the most abundant nonprotein thiol in cells.[3,15,16] The rate limiting steps in the biosynthesis of GSH are

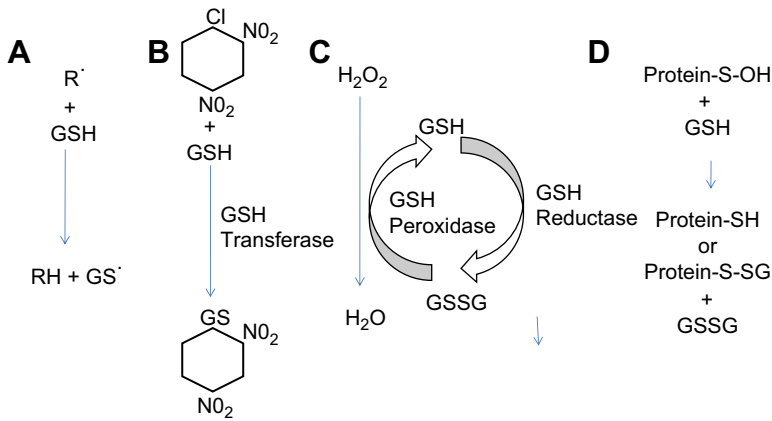

Fig. 1. Major antioxidant mechanisms in the liver involving glutathione (GSH). (*A*) GSH can nonenzymatically act directly on free radicals such superoxide radical, hydroxyl radical, nitric oxide, and carbon radical and aid in their removal. (*B*) Cytosolic and mitochondrial GSH transferases can catalyze the nucleophilic attack by reduced GSH on nonpolar compounds that contain an electrophilic carbon, nitrogen, or sulfur atom. (*C*) In conjunction with GSH peroxidase, GSH can remove hydro- and organic peroxides from the cell. The resultant oxidized glutathione disulfide (GSSG) is reduced back to GSH by GSH reductase. (*D*) GSH can help to maintain protein sulfhydryl groups by nonenzymatically reducing protein sulfenic acids groups or forming protein-GSH mixed disulfides.

the availability of cysteine and the activity of the first enzyme in the biosynthetic pathway, γ-glutamylcysteine synthetase (GCS) (**Fig. 2**). Cysteine is derived from the diet and the breakdown of protein. The liver also has the unique ability to enzymatically convert methionine to cysteine via the transsulfuration pathway (**Fig. 3**). The short half-life of GSH (2–3 hours) and the requirement for dietary cysteine make hepatic GSH levels highly dependent on nutritional conditions. Starvation for 48 hours results in 50% to 75% reductions in hepatic GSH levels in normal rats, which normalize within a few hours of refeeding.[15] Hepatocyte GSH is synthesized in the cytosol and transported into intracellular organelles, particularly the mitochondria.[15,16] GSH transport across the mitochondrial membrane is vital in maintaining mitochondrial defense against oxidant injury. Mitochondrial GSH depletion is an early event in many forms of toxic liver injury and sensitizes hepatocytes to cell death.[17]

GSH serves several vital defense mechanisms in the liver. It exists principally in two forms—a thiol reduced form (GSH) and a disulfide oxidized form (GSSG).[15,16] Normally, the ratio of GSH to GSSG is about 95:1 and is maintained by GSH redox cycling (see **Fig. 1**). The GSH redox cycle involves the oxidation of GSH (GSSG) catalyzed by GSH peroxidase and the recovery of GSH by reduction of GSSG catalyzed by GSH reductase. During this redox recycling, GSH can remove hydrogen and organic peroxides from the cell. Conjugation and removal of endogenous and exogenous toxins from the cell through a family of glutathione S-transferase via the mercapturic acid pathway is another important cytoprotective pathway for GSH. Reduced GSH can also nonenzymatically react with free radicals, including superoxide radicals, hydroxyl radicals, and nitric oxide. In addition, GSH can aid in the reduction of other antioxidants such as tocopherol and ascorbate. GSH also maintains protein sulfhydryl groups. GSH can reduce protein sulfenic acids and bind to sulfhydryl groups to form protein-GSH mixed disulfides (see **Fig. 1**).[15,16]

Vitamin E (α-tocopherol) is an essential nutrient derived from food and nutritional supplements. Vitamin E is considered the predominant lipid-soluble antioxidant protecting membrane phospholipids from lipid peroxidation. Ascorbate (vitamin C) is a water-soluble reducing agent that protects against ROS such as hydrogen peroxide. When vitamin E works as an antioxidant, it is oxidized to a potentially harmful radical, which needs to be reduced back to α-tocopherol by vitamin C.[18]

CYTOPROTECTIVE AGENTS

S-Adenosylmethionine

S-adenosylmethionine (SAMe) is generated from L-methionine and ATP in a two-step reaction catalyzed by methionine adenosyltransferase (MAT) (see **Fig. 3**).[7,12] MAT activity is decreased in many types of liver disease, resulting in decreased hepatocellular levels of SAMe.[7,12,19–21] Reactive oxygen and nitrogen species inactivate MAT secondary to oxidation or nitrosylation of a cysteine residue on the enzyme.[22]

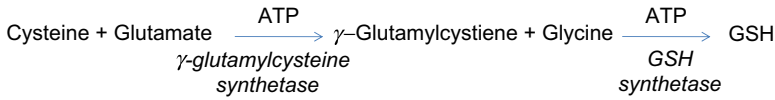

Fig. 2. Biosynthesis of glutathione. Glutathione (GSH) is synthesized from three amino acid precursors: cysteine, glutamate, and glycine. The rate limiting step in its formation is the availability of cysteine which, in the liver, is largely dependent on dietary sources of cysteine and methionine.

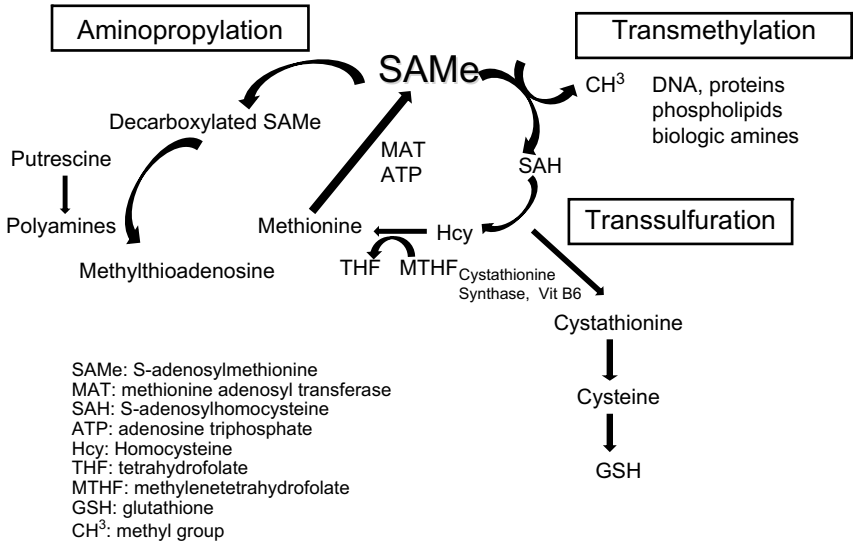

Fig. 3. Hepatic SAMe metabolism. SAMe is generated from methionine in a reaction catalyzed by MAT. The methyl group of SAMe is donated in transmethylation reactions, and the propylamino group is donated for polyamine synthesis (aminopropylation) with MTA being a by-product of this reaction. SAH is a by-product of transmethylation reactions and is hydrolyzed to form homocysteine and adenosine through a reversible reaction catalyzed by SAH hydrolase. Homocysteine can be remethylated to form methionine or can undergo the transulfuration pathway to form cysteine.

Inactivation can be reversed by increasing GSH or overcome by exogenous administration of SAMe.

Within the liver, SAMe is metabolized via three pathways—transmethylation, transulfuration, and aminopropylation (see **Fig. 3**).[7] Most of the SAMe generated is used in transmethylation reactions (85%). In these reactions, SAMe donates its methyl group to a variety of compounds, including nucleic acids, proteins, phospholipids, and biologic amines in reactions catalyzed by methyltransferases. The by-product of these transmethylation reactions, S-adenosylhomocysteine (SAH), is subsequently hydrolyzed to homocysteine and adenosine by SAH hydrolase. Homocysteine is then either remethylated to methionine or undergoes transulfuration. Remethylation requires two enzymes, methionine synthase and betaine methyltransferase, folate, and vitamin B_{12}. In the transsulfuration pathway, homocysteine is converted by a series of enzymatic steps in the presence of vitamin B_6 to cysteine. Cysteine can then be used to form GSH (see **Fig. 2**).

The aminopropylation pathway results in the decarboxylation of SAMe, with transfer of its aminopropyl group first to putrescine and then to spermidine to form polyamines.[7] Methyladenosine (MTA) is an important intermediate in this pathway. Exogenous SAMe can also spontaneously convert to MTA, which is stable and can freely pass through cell membranes.

The cytoprotective benefits of SAMe in hepatobiliary disease include (1) augmentation of hepatocyte GSH levels, (2) improvement in membrane fluidity, (3) modification of cytokine expression, (4) alterations in DNA/histone methylation, and (5) modulation of apoptosis (**Table 1**).[7,20,21]

Table 1
Cytoprotective agents

Cytoprotective Agent	Mechanisms of Action	Dose	Side Effects/ Drug Interactions	Indication
S-adenosylmethionine	Antioxidant: increases GSH Stabilization of membrane function Modulation of cytokine expression Anti-apoptotic in normal cells	20 mg/kg/d PO Food decreases bioavailability	No side effects	Necro-inflammatory hepatopathies Metabolic hepatopathies (FHL) Cholestatic hepatopathies APAP toxicity
N-acetylcysteine	Antioxidant: increases GSH Anti-inflammatory Improves hepatic microcirculation Improves tissue oxygen delivery	140 mg/kg IV once, then 70 mg/kg IV q 6 h (APAP and ALF) 100 mg/kg/24 h CRI (ALF)	Vomiting with oral preparations	APAP toxicity ALF
Ursodeoxycholic acid	Replaces hepatotoxic bile acids Choleretic Anti-apoptotic Immunomodulatory Stabilizes mitochondria	10–15 mg/kg/d PO Food enhances absorption Increase dose (BID) with severe cholestasis	Rarely vomiting May increase bioavailability of vitamin E and cyclosporine	Cholestatic hepatopathies Necro-inflammatory hepatopathies Metabolic hepatopathies Immune-mediated hepatopathies
Silymarin	ROS scavenger Anti-inflammatory Antifibrotic Increases hepatic protein synthesis Choleretic	Silymarin: 20–50 mg/kg/d divided q 6–8 PO Siliphos: 3–6 mg/kg/d PO	No side effects Inhibits the activity of drug metabolizing enzymes	*Amanita* mushroom toxicity Hepatotoxicity Cholestatic hepatopathies Necro-inflammatory hepatopathies
Vitamin E	Antioxidant Anti-inflammatory	10–15 IU/kg/d PO of α-tocopherol acetate	None May inhibit the absorption of other fat-soluble vitamins when administered at high doses	Cholestatic hepatopathies Necro-inflammatory hepatopathies

Abbreviations: ALF, acute liver failure; APAP, acetaminophen; FHL, feline hepatic lipidosis; GSH, glutathione.

Because SAMe increases the intracellular availability of cysteine, it increases hepatic GSH levels and has antioxidant activity.[12,19–21] Several studies have demonstrated that SAMe can alleviate signs of oxidative and nitrosative stress.[12,19] Particularly striking is the ability of SAMe to reverse signs of mitochondrial damage.[7] Alterations in membrane fluidity, especially in the mitochondrial membrane, accompany hepatobiliary disease. SAMe restores normal membrane fluidity via methylation of mitochondrial membrane phospholipids, thereby re-establishing normal mitochondrial GSH transport.[7,17]

SAMe modulates cytokine expression.[7,12] SAMe inhibits lipopolysaccharide-stimulated release of TNF-α from macrophages. This anti-inflammatory activity is due to inhibition of histone methylation of the TNF-α promoter by SAMe or its by-product, MTA.[22] SAMe also increases the production of anti-inflammatory cytokines such as IL-10.[7]

SAMe regulates hepatocyte apoptosis.[7] It protects normal hepatocytes against okadaic acid and bile acid induced apoptosis.[7,23] MTA recapitulates these actions.[7] In liver cancer cell lines, SAMe is pro-apoptotic.[7,24] One study has linked this disparity to differential activation of the pro-apoptotic kinase, JNK.[24]

SAMe also has growth regulatory effects on hepatocytes. Mice with genetic depletion of MAT develop hepatic hyperplasia, have abnormal hepatic regeneration after partial hepatectomy, and eventually develop hepatocellular carcinoma.[20] SAMe levels are low in proliferating and regenerating hepatocytes and high in quiescent hepatocytes. SAMe inhibits proliferation in hepatoma cells and protects against the development of neoplastic foci in hepatotoxic models of hepatocarcinogenesis. The growth inhibitory effects of SAMe are due in part to inhibition of the mitogenic response to hepatocyte growth factor.[7,20] SAMe is antiproliferative and pro-apoptotic in neoplastic hepatocytes, whereas it is mitogenic and anti-apoptotic in normal hepatocytes.

Literature review

The effects of SAMe administration in hepatobiliary disease have been evaluated in animal models and human clinical trials.[7,12,19,20,25–28] SAMe improves survival in animal models of alcohol, acetaminophen, galactosamine, and thioacetarsamide induced hepatotoxicity and in ischemia-reperfusion induced liver injury. SAMe treatment also decreases fibrosis in rats treated with carbon tetrachloride. SAMe has also shown efficacy in ameliorating hepatic steatosis. Mice with a genetic depletion in MAT develop hepatic steatosis, implying a link between normal SAMe levels and lipid metabolism. In a genetic model of hepatic lipidosis using ob/ob (leptin deficient) mice, SAMe administration reduced triglyceride accumulation as well as apoptosis during treatment with the hepatotoxin pyrazole.[25] In human patients with established steatosis and in patients with chronic hepatitis given prednisone who normally develop steatosis, SAMe administration attenuated or prevented fat accumulation, respectively.

Several human clinical trials with SAMe have been conducted in Europe. A meta-analysis of six placebo-controlled clinical trials evaluating parenteral SAMe administration in acute intrahepatic cholestasis confirmed that it decreased serum bilirubin levels and ameliorated histologic evidence of hepatocellular necrosis.[26] A follow-up study with oral SAMe confirmed continued beneficial effects on biochemical parameters and clinical signs.[27] In human patients with alcoholic liver cirrhosis, long-term oral supplementation with SAMe (2-year period at 1200 g/d) increased survival and decreased the need for liver transplantation in a subgroup of patients with less advanced disease.[28]

Oral SAMe administration has been evaluated in healthy cats and dogs. Normal dogs and cats given 20 mg/kg and 30 to 50 mg/kg orally, respectively, had significantly increased plasma SAMe concentration, increased hepatic GSH levels, and no overt signs of toxicity.[29,30] Additionally, in cats, a decreased concentration of red blood cell thiobarbituate-reacting substances (oxidative membrane products known as TBARs) and increased resilience to osmotic challenge suggested that chronic SAMe treatment may have antioxidant and membrane-stabilizing effects in erythrocytes.[29] Oral SAMe administration ameliorated acetaminophen-induced red blood cell and hepatic damage in cats (85 mg/kg for 3 days followed by 40 mg/kg) and dogs (40 mg/kg followed by 20 mg/kg), respectively.[31,32] In a study of prednisolone-treated dogs, oral SAMe (20 mg/kg) did not prevent the development of hepatic vacuolar changes or the induction of serum hepatic enzyme activity but increased hepatic GSH levels.[30] Neither prednisolone nor SAMe plasma concentrations were altered by coadministration.[30]

Dose and pharmacokinetics

In the United States veterinary market there are two commercially available stable salts: a 1,4-butanediosulfonate salt (Denosyl-SD4, Nutramax Laboratories, Edgewood, Maryland) and a tosylate salt (Zentonil, Vetoquinol, Buena, New Jersey). Tablets are blister packed because the salts are hydroscopic and enteric coated to prevent inactivation in stomach acid. The tablets must not be split or crushed. Food interferes with absorption, requiring that SAMe be given on an empty stomach. The recommended oral dose is 20 mg/kg/d. SAMe has a high hepatic first-pass effect and a low bioavailability (approximately 3%). Measurable plasma and tissue concentrations are obtained, even within the cerebrospinal fluid in the cat and dog.[33] Maximal plasma concentrations are variable but generally are achieved between 1 and 4 hours in dogs and 2 and 8 hours in cats.[29,30] SAMe is rapidly metabolized intracellularly, with the portion not metabolized undergoing renal or fecal excretion.[3]

Side effects and drug interactions

SAMe is well tolerated. Reported side effects include immediate post pill nausea, food refusal, and anxiety.[3] In a few cats, post-dosing emesis has necessitated discontinuation of treatment.[3]

Use in canine and feline hepatobiliary disease

The therapeutic potential of SAMe in companion animals with hepatobiliary disease is largely unknown because no clinical studies have been published. Dogs and cats with naturally occurring liver disease have reductions in hepatic GSH levels. In one study, low hepatic GSH levels were present in 42% and 33% of dogs with necroinflammatory or vacuolar hepatopathies, respectively[34]; however, some dogs and cats with liver disease also had increased GSH levels. In a separate study, dogs with copper toxicosis, extrahepatic cholestasis, and chronic hepatitis had low hepatic GSH levels, and many had concurrent decreases in hepatic mRNA levels for SOD and catalase.[35] Low hepatic GSH levels were present in 80% and 75% of cats with necroinflammatory liver disease and hepatic lipidosis, respectively.[34] Considering the pivotal role of oxidant stress in liver injury, therapies aimed at restoring normal redox balance by normalizing GSH levels would be expected to be of benefit.

Based on animal models, human clinical trials, and what is known about hepatobiliary disease in companion animals, SAMe administration would likely be beneficial in a variety of disorders in dogs and cats. These disorders would include acute intrahepatic or extrahepatic cholestatic disorders (eg, cholecystitis, gallbladder mucocele, choledochitis), necroinflammatory diseases (eg, canine chronic hepatitis, feline

cholangitis), metabolic diseases (eg, canine vacuolar hepatopathy, feline hepatic lipidosis), and toxic (eg, acetaminophen) or ischemic hepatopathies.

N-Acetylcysteine

N-acetylcysteine (NAC) is a stable formulation of the amino acid L-cysteine that can be given parenterally to replenish intracellular cysteine and GSH levels. It is a well-recognized antidote for acetaminophen-induced red blood cell and hepatocyte toxicity. NAC also has a myriad of other cytoprotective effects, including an effect on vascular tone that may improve oxygen delivery in acute liver failure (ALF), an ability to enhance hepatic mitochondrial energy metabolism, and anti-inflammatory actions (blocks polymorphonuclear cell (PMN)–endothelial cell adhesion, PMN activation, and cytokine release (TNF-α)).[36]

Literature review

NAC has been evaluated in animal models of hepatotoxicity, ALF, ischemia-reperfusion injury, bile duct obstruction, and cirrhosis. NAC is beneficial in the treatment of several hepatotoxins, acetaminophen being the best studied.[36] Additionally, NAC has been evaluated in cyclosporine A, arsenic, azathioprine, and lipopolysaccharide-mediated liver injury.[37–40] The cytoprotective effect of NAC in these hepatotoxic models is mediated by its ability to increase hepatic GSH levels.

In ALF, administration of NAC improves vasomotor tone in peripheral, cerebral, and sinusoidal vascular beds. Its effects include increased mean arterial pressure, improved oxygen extraction in capillary beds, and decreased cerebral perfusion pressure.[41,42] In ischemia-reperfusion injury, improvements in the hepatic microcirculation and decreased hepatic damage have been seen with NAC pretreatment in rabbit and rodent models, although no benefits were seen in a recent canine study.[43–46] In bile duct ligated rats and dogs, NAC improves portal and hepatic microcirculatory blood flow.[47,48] The effects of NAC on the microcirculation are believed to be related primarily to its antioxidant effects, because ROS are a major stimulus for inflammatory cell recruitment to endothelial cell surfaces.

NAC is currently undergoing human clinical trials in the treatment of non-acetaminophen associated ALF.[49,50] In a retrospective study in children with ALF, a 72-hour infusion of NAC significantly improved survival when compared with placebo.[49] In preliminary reports of a randomized blinded clinical trial in pediatric patients with ALF, NAC infusion was associated with a shorter length of hospital stay, a higher incidence of native liver recovery without transplantation, and a better survival after transplantation.[50] Currently, a similar clinical trial is ongoing in adult patients with ALF.

Dose and pharmacokinetics

In veterinary medicine, NAC is considered to be the antidote of choice for acetaminophen toxicity in small animals. NAC restores intracellular GSH levels that aid in the detoxification of the reactive intermediate generated by CP-450 metabolism of acetaminophen. There are several published studies on the use of NAC in acetaminophen toxicity in the dog and cat.[51–54] NAC is most effective if given within 8 to 16 hours of ingestion; however, in human patients, treatment up to 53 hours after drug ingestion has yielded a positive clinical response. An initial dose of 140 mg/kg is followed by doses of 70 mg/kg intravenously every 6 hours for seven treatments. In human ALF, a constant rate infusion dose of 100 mg/kg/24 h is being evaluated therapeutically. NAC should be administered through a nonpyrogenic filter (0.25 μm) using a 10% solution diluted 1:2 or more with saline. The high incidence of gastric irritation and vomiting

limits the oral use of NAC, which is rarely indicated given the availability of oral SAMe for GSH replenishment.

Side effects and drug interactions

Side effects of an intravenous bolus in humans include gastrointestinal upset, allergic reactions, and hemodynamic changes (increased or decreased blood pressure). Allergic reactions are rare but may manifest as rash, pruritus, angioedema, or bronchospasm. Parenteral NAC administration appears to be well tolerated in veterinary patients, with no adverse events reported in the literature. The oral median lethal dose (LD_{50}) in dogs is greater than 1000 mg/kg.

Use in canine and feline hepatobiliary disease

Indications for NAC use in veterinary medicine include acetaminophen toxicity, Heinz body anemia, suspected toxin-related liver injury, and ALF (regardless of underlying etiology). Of particular interest is the use of NAC in ALF caused by suspected toxicosis (eg, carprofen and trimethoprim toxicity in dogs, diazepam and methimazole toxicity in cats) and in cats with severe hepatic lipidosis (given their propensity for Heinz body anemia and oxidant injury). Given that liver transplantation is not an option in veterinary medicine and that NAC in human ALF improves native liver survival, the use of NAC should be considered in any veterinary patient fitting the criteria for ALF.

Ursodeoxycholic Acid

Yutan, a Chinese compound derived from the dried bile of the Chinese black bear, has been used for centuries for its hepatobiliary healing powers. In 1936, ursodeoxycholic acid (UDCA) was identified as the major bile acid responsible for Yutan's hepatoprotective effects.[4] Currently, synthetic forms of UDCA are approved for the treatment of hepatobiliary disease in human patients (Actigal, Novartis, Basel, Switzerlandand URSO, Axcan, Mont-Saint-Hilaire, Canada).

Bile acids are organic anions synthesized exclusively in the liver from cholesterol. The rate-limiting step is the addition of a hydroxyl group in the seventh position of the cholesterol steroid nucleus. Because cholesterol already contains a 3α-OH group, the simplest bile acids are the 3α, 7α di-OH bile acids such as chenodeoxycholate. Additional hydroxylation at the 12α position creates the tri-OH bile acids in the cholate group. The primary bile acids, cholate and chenodeoxycholate, are conjugated in the liver to either glycine or taurine in the dog or exclusively to taurine in cats.[3] The major circulating bile acid in both species is taurocholate. Some bile acids are hepatotoxic. The elements of toxicity are not completely understood but correlate to some degree with hydrophobicity. In general, the more hydroxylated the bile acid, the less hydrophobic and the less toxic. The more hydrophobic bile acids damage hepatocytes primarily by disrupting mitochondrial membranes and activating apoptotic death receptors.[4,55]

UDCA is a cytoprotective hydrophilic dihydroxylated bile acid even though it is almost structurally identical to the much more hepatotoxic bile acid, chenodeoxycholate. The unique cytoprotective action of UDCA is still not fully understood but several mechanisms have emerged, including replacement of more toxic bile acids in the bile acid pool, stimulation of choleresis, anti-apoptotic effects, stabilization of mitochondrial function, and immunomodulatory actions.[4,55] The cytoprotective effects of UDCA are not limited to hepatobiliary cells but have been demonstrated in cardiac myocytes, neuronal cells, and gastrointestinal epithelial cells.[56–58]

Because serum and hepatic retention of bile acids accompanies most hepatobiliary disorders, and because some bile acids are potentially hepatotoxic, they likely

contribute to the pathologic progression of cholestatic hepatopathies. One mechanism of UDCA cytoprotection is replacement of more hydrophobic hepatotoxic bile acids in the bile acid pool[3,4,55]; however, this view is overly simplistic, because a correlation between UDCA enrichment of the bile acid pool and its therapeutic effect has been hard to demonstrate. In addition, this effect would be of limited value in dogs and cats because the major circulating bile acid is the relatively nontoxic taurocholate.

UDCA stimulates choleresis, which increases the elimination of endogenous toxins normally excreted in the bile, such as copper, leukotrienes, and bilirubin. Choleresis is the result of an increase in the expression of membrane transporters necessary to generate bile flow and direct stimulation of a bicarbonate-rich bile flow in the bile ducts.[4,55] The secretory capacity of a hepatocyte is dictated by the number and activity of transporter proteins located on its membrane. UDCA increases the canalicular membrane expression of several transporters, including the bile salt excretory pump, BSEP, and the organic anion pump, MRP2. UDCA stimulates the insertion of transporter molecules that are sitting in intracellular endosomal compartments into the membrane.[4] UDCA also increases cholangiocyte excretion of bicarbonate-rich fluid.[4,55] By increasing intracellular Ca^{+2} in cholangiocytes, UDCA activates both Ca^{+2}–dependent Cl^- channels and Cl^-/HCO_3^- exchange.[4]

A major cytoprotective action of UDCA lies in its ability to inhibit apoptosis. UDCA modulates the apoptotic threshold by preserving mitochondrial integrity.[4,57] In cultured hepatocytes, UDCA protects against the loss of mitochondrial membrane potential, the onset of the MOMP, and the loss of cytochrome C, and can decrease the production of ROS that accompanies apoptosis due to various stimuli. UDCA can increase mitochondrial GSH levels by stabilizing mitochondrial membrane transporters or by increasing the activity of MAT. Furthermore, UDCA can induce the expression of the anti-apoptotic mitochondrial protein, Bcl-2, and decrease Bax levels. Additionally, UDCA can promote survival by controlling transcription factors and kinases involved in apoptosis. In primary hepatocytes, UDCA inhibits TGF-β–induced apoptosis by preventing activation of an E2F-1/p53 transcription factor–induced apoptotic pathway. In hepatocytes, neurons, and cardiac myocytes, UDCA cytoprotection requires activation of the lipid prosurvival kinase, phosphoinositide-3-kinase, and its downstream mediator, Akt. UDCA can also act as a molecular chaperone and modulates apoptosis associated with endoplasmic reticulum stress.[59]

UDCA has immunodulatory properties. UDCA down-regulates aberrant major histocompatibility complex expression on hepatobiliary cells induced by cholestasis.[4] This aberrant expression renders the cells more vulnerable to immune targeting by activated lymphocytes, leading to cell damage.[4,55] UDCA also suppresses interleukin-2 and 4 production. Studies show that UDCA can directly activate the glucocorticoid receptor by interacting with a region of the receptor distinct from the cortisol binding site. UDCA binding to the glucocorticoid receptor induces nuclear translocation of the glucocorticoid receptor and suppresses activation of NF-κβ.[60,61] Inhibition of the UDCA–glucocorticoid receptor interaction prevents UDCA's cytoprotective effect in cultured hepatocytes.[60]

Literature review

UDCA use has been evaluated extensively in animal models of hepatotoxicity. In bile duct ligated rats, UDCA prevents GSH depletion (by up-regulating GCS) and prevents changes in mitochondrial membrane potential and lipid content that accompany chronic cholestasis.[4,55,57] In vivo feeding of toxic bile acids to rats causes apoptosis, whereas simultaneous treatment with UDCA inhibits this effect and prevents the movement of Bax to the mitochondria. In an endoplasmic reticulum stress model of

apoptosis in obese diabetic mice, oral UDCA therapy inhibited the up-regulation of endoplasmic reticulum stress markers in the liver and adipose tissue of mice. UDCA can also inhibit hepatocyte triglyceride accumulation in a rat model of hepatosteatosis.[62]

UDCA is cytoprotective in myocytes and neuronal cells. Administration of UDCA in rats just before inducing myocardial infarction results in a reduction in the number of apoptotic myocytes and the area of infarction in a comparison with controls.[56] UDCA also prevents neuronal cell apoptosis in experimental models of Alzheimer's and Huntington's disease in mice.[57]

In humans, UDCA is used to treat a variety of cholestatic hepatopathies, including primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), pediatric cholestatic disorders, cystic fibrosis, and intrahepatic cholestasis associated with pregnancy and prolonged total parenteral nutrition.[63–68] PBC is an immune-mediated, chronic inflammatory hepatobiliary disease that usually results in biliary cirrhosis and eventual liver failure. UDCA is the only approved treatment for PBC even though its effect on long-term survival remains uncertain.[63–65] Although meta-analyses have failed to show benefit in terms of survival or transplantation rates, many individual studies have shown significant improvements in biochemical parameters, clinical signs, and histologic scores, and, in isolated cases, survival benefits.[63–65] Considering the complexities of PBC (eg, its variability in disease progression and the long natural history of the disease) and issues with study design (eg, studies of insufficient duration, small patient numbers, inconsistently defined end points, and suboptimal UDCA dosing), meta-analyses may fail to recognize beneficial effects of UDCA. In PSC, UDCA improves serum liver chemistries and bilirubin but has no effect on disease progression or transplant-free survival.[66] Similar to the studies on PBC, even the largest of these studies was probably too small and the follow-up period too short to allow evaluation of survival. UDCA has proven benefit in children who have genetic abnormalities causing severe cholestasis, for managing bile flow aberrations in cystic fibrosis, and as adjunctive therapy in immunomodulatory protocols in humans who have immune-mediated liver disease.[55,67] UDCA reduces cholestasis, improves serum aminotransferase levels, and decreases pruritus in women with intrahepatic cholestasis related to pregnancy.[68] There is some evidence for an additive effect of concurrent SAMe and UDCA administration in this disease.[68]

Little information is available in the literature in regards to UDCA use in small animals. In a single case report, a dog with chronic hepatitis was given 15 mg/kg/d of UDCA, which resulted in enrichment of UDCA in the serum associated with biochemical (decreases in alanine aminotransferase, alkaline phosphatase, cholesterol, and bilirubin) and clinical improvement (increased appetite and energy level).[69] In four normal cats, 15 mg/kg/d of UDCA for 8 weeks resulted in no adverse clinicopathologic effects.[70]

Dose and pharmacokinetics

The pharmacokinetics of UDCA have been examined in dogs, humans, and rodents. UDCA is absorbed passively, primarily in the small intestine. Absorption is enhanced in the presence of food. Greater than 60% of the oral dose undergoes hepatic uptake where it is conjugated with taurine or glycine and then undergoes enterohepatic circulation. UDCA that escapes enterohepatic circulation is metabolized to lithocholate in the colon and eliminated in the feces or urine. A dose of 15 mg/kg/d has been extrapolated from human medicine. Bioavailability decreases with advanced cholestasis due to decreased absorption, decreased hepatic extraction, and increased renal

elimination; therefore, twice daily administration may be necessary in severe cholestatic disease.

Side effects and drug interactions

UDCA is well tolerated. Little or no toxicity has been seen in human patients. The only side effect appears to be diarrhea. Similarly, in small animals, rare side effects include vomiting and diarrhea. Extensive toxicologic studies performed in healthy dogs for Food and Drug Administration approval did not reveal any serious side effects. No adverse effects were noted in normal cats at doses ranging from 10 to 15 mg/kg/d for treatment periods ranging from 2 to 3 months.[70] UDCA treatment may increase the bioavailability of cyclosporine and vitamin E. Concurrent use of aluminum-containing antacids may decrease the absorption of UDCA.[3]

Use in canine and feline hepatobiliary disease

UDCA should be considered as ancillary therapy in a variety of acute and chronic hepatopathies in the dog and cat. In acute hepatobiliary disease, it would be useful as a choleretic agent to treat intra- and extrahepatic cholestasis in the absence of complete bile duct obstruction. Its use could also be considered in animals assessed as having a large amount of hyperechoic sludge in their gall bladders on ultrasound. Additionally, UDCA could be used in asymptomatic or mildly symptomatic patients with gall bladder mucoceles, in the absence of bile duct obstruction, that are poor surgical candidates due to concurrent disease.[71]

Owing to its anti-apoptotic and immunomodulatory actions, UDCA would likely be beneficial in chronic necroinflammatory hepatobiliary disease regardless of etiology. Because bile acid concentrations are particularly high in the bile ducts, UDCA may be particularly beneficial in cats with cholangitis. In dogs, ancillary UDCA treatment would likely be of benefit in cases of chronic hepatitis (breed related [including primary copper storage hepatopathies], infectious, or immune mediated). In some chronic hepatopathies, particularly where corticosteroids are contraindicated, UDCA may be indicated as part of the immunosuppressive regimen. The therapeutic effects of UDCA may be enhanced by concurrent SAMe administration; it is unclear whether this effect may be synergistic or additive.[68] There is some evidence both in vitro and in vivo that the actions of SAMe and UDCA may be additive.[68,72]

Silymarin

The major active ingredient of benefit in milk thistle is silymarin. Silymarin is actually a group of several closely related flavinoids but consists primarily of four isomers: silybin, isosilybin, silydianin, and silychristin. Silybin is the major active component of silymarin.[6,9,10,12]

Silymarin has several beneficial actions useful in the treatment of hepatobiliary disease, including antioxidant, anti-inflammatory, and antifibrotic properties.[6,10,12] Additionally, silymarin has an ability to modulate hepatocyte transport and increase hepatic protein synthesis.[6] Silymarin acts as an antioxidant by reducing free radical production and lipid peroxidation.[6] Silymarin also scavenges ROS and protects against GSH depletion.[6,12] Anti-inflammatory properties include suppression of NF-$\kappa\beta$ activation, inhibition of TNF-α induced cytotoxicity, and increased expression of IL-10.[6,12,73] Silymarin has an inhibitory effect on the 5-lipoxygenase pathway resulting in inhibition of leukotriene synthesis.[6] It has also been shown to protect hepatocytes against T cell–induced injury and inhibits hepatitis C viral replication.[73] Silymarin also exhibits antifibrotic actions, including inhibition of TGF-β secretion and stellate cell activation.[10]

Silymarin modulates hepatocyte transport.[6,10,12] It promotes choleresis by increasing insertion of transporters into the apical membrane of hepatocytes. Additionally, it inhibits hepatocyte uptake of phalloidin, the toxic agent resulting in ALF secondary to *Amanita* mushroom ingestion. Silymarin increases protein synthesis through stimulation of ribosomal RNA polymerase in hepatocytes.[9] Silymarin also accelerates hepatocellular regeneration as a result of increased gene transcription/translation and enhanced DNA biosynthesis.

Literature review

Substantial in vitro and in vivo evidence suggests that silymarin can protect the liver from a wide variety of toxins, including acetaminophen, ethanol, carbon tetrachloride, aflatoxin, and *Amanita phalloides* toxins, as well as from ischemic, viral, and radiation-induced injury.[6,11,74–77] Antifibrotic properties have been demonstrated in bile duct ligated rats and alcohol fed baboons, in which stellate cell proliferation and collagen production were inhibited.[78,79] Silymarin can also augment the synthesis of DNA, RNA, and protein following partial hepatectomy in rats.[80]

In humans, silymarin use has been evaluated in a variety of liver conditions, including alcoholic liver disease, liver cirrhosis, acute and chronic viral hepatitis, *A phalloides* poisoning, and toxic and drug-induced liver diseases.[6,9,10] Evidence from these studies to support silymarin use in human hepatobiliary disease is conflicting. Some studies have shown improvements in serum transaminases and bilirubin, clinical signs and indicators of oxidative damage, whereas others have failed to show any benefit. The benefits of silymarin therapy in these studies are difficult to interpret and compare due to different methodologies, small sample sizes, inclusion of patients with heterogeneous hepatic pathology, ill-defined end points, variable treatment durations, and the use of nonstandardized products and dosages that are not well justified. Nevertheless, because of the overwhelming evidence for its cytoprotective effects in in vitro and in vivo models, a meeting (sponsored jointly by the National Institute of Diabetes and Digestive and Kidney Disease, the National Center for Complementary and Alternative Medicine, and the National Institute on Alcohol Abuse and Alcoholism) was held in 2004 with two objectives in mind[81]: (1) to develop a standardized reliable silymarin product that could be used in clinical trials, and (2) to plan and initiate clinical trials of this product in liver disease, with a particular focus on nonalcoholic steatohepatitis (NASH) and hepatitis C. Several phase I and II clinical trials as a direct result of this meeting are nearing completion.

In the veterinary literature, silymarin has been evaluated for its protective action against carbon tetrachloride and phalloidin toxicity. A single study found silymarin to be protective against carbon tetrachloride toxicity in dogs.[77] Silybin was found to be completely protective against *A phalloides* intoxication in Beagles at a dose of 50 to 150 mg/kg when given 5 to 24 hours after ingesting an LD_{50} dose of *A phalloides*.[82] Data from an uncontrolled study suggest that the use of silymarin up to 48 hours after ingestion is effective in preventing severe hepatotoxicity.[9] Silymarin prevents binding of the phalloidin toxin to hepatocytes and interrupts the enterohepatic circulation of the toxin.

Dose and pharmacokinetics

Silymarin's bioavailability is low due to erratic absorption from the gastrointestinal tract. It has a short plasma half-life but is preferentially accumulated in the liver.[81] It is excreted in bile as a glucoronide and sulfoglucoronide conjugate and undergoes some enterohepatic circulation. Bile concentrations are 100 times those seen in serum.[3]

The dose required to achieve a therapeutic range in small animal patients is unknown. In human medicine, variable dosing regimens have been used, with doses of 50 to 150 mg/kg given in cases of *A phalloides* intoxication and 7 to 15 mg/kg/d in cases of hepatitis. Extrapolation of the dose from human studies suggests that 20 to 50 mg/kg/d of silymarin divided into three to four doses per day may be of therapeutic value.[81] Therapeutic formulations should contain 70% to 80% silymarin. Unfortunately, despite labeling claims, there are significant variations between commercially available products and no assurance of extract purity.[81]

More recently, a new product (initially called IdB1016) has become available called Siliphos.[6] Siliphos, a formulation of silibin complexed with phosphatidylcholine, is three to five times more bioavailable than silymarin[83,84]; values in rodent studies have shown concentrates in bile 10 times greater with the phosphatidylcholine complexed compound.[85] These results suggest that this compound can be dosed at 3 to 6 mg/kg/d.[83,84] Commercially it is available as a combined product with vitamin E and zinc (Marin, Nutramax, Edgewood, Maryland) or SAMe (Denamarin, Nutramax, Edgewood, Maryland). A recent study demonstrated that this product increased silybin blood levels in dogs, with a peak maximum at 3 hours and restoration of baseline values by 24 hours.[83] A preliminary pharmacokinetic study of Siliphos in normal cats at a dose of 10 mg/kg orally found a bioavailability of 6% to 7%.[86] A separate study demonstrated that this dose increased neutrophil GSH content and neutrophil function as measured by maximal phagocytic and oxidative burst activity.[87]

Side effects and drug interactions

Silymarin is reported to have an extremely low toxicity and has been used extensively in human clinical patients with few reported side effects. Similarly, no serious adverse effects have been reported in animal studies. Mild side effects such as gastrointestinal upset, pruritus, and headache have been rarely reported in humans. The silybin-phosphatidylcholine complex has been evaluated in both acute and chronic use safety studies in dogs. An acute toxicity study in dogs using levels greater than 80 times the amount in Marin revealed no adverse effects. In a chronic toxicity study in monkeys who received a similar dose for 26 weeks, no side effects were seen. Silymarin has also been evaluated in normal cats and found to have no clinical outward signs of toxicity in a preliminary pharmacokinetic study when given at a dose of 10 mg/kg.[86]

Silymarin inhibits the activities of glucoronide transferases, some cytochrome P450 enzymes, and P-glycoprotein. Inhibition of cytochrome P450 enzymes in vitro only occurred at concentrations greatly exceeding physiologically reachable ones.[6] Nevertheless, given the possibility of silymarin use interfering with the metabolism of other drugs, drug interactions should be considered in polymedicated patients.[3,6]

Use in canine and feline hepatobiliary disease

Silymarin may have benefit in cases of hepatotoxicity, hepatobiliary disease associated with cholestasis, and chronic hepatopathies. Silymarin use is indicated in the treatment of *Amanita* mushroom toxicity, although high doses are needed to inhibit uptake of the phalloidin toxin, and intravenous formulations (ie, silybin dihemisuccinate) are not currently available for clinical use in the United States.

In acute and chronic cholestasis, silymarin may promote choleresis and prevent proinflammatory and profibrotic complications associated with the retention of endogenous toxins normally excreted in bile. Given its anti-inflammatory, antioxidant,

antifibrotic, and anti-apoptotic effects, silymarin administration should be considered in dogs and cats with chronic necroinflammatory hepatobiliary disease.

Vitamin E

Vitamin E is an essential nutrient derived from food and nutritional supplements. The vitamin E family consists of eight highly lipophilic antioxidant compounds widely distributed in plants. Alpha-tocopherol is the most bioavailable and active form of vitamin E.[1–3,5,12]

Vitamin E has a major role in the protection of membrane phospholipids from oxidative damage. It defends against peroxidative membrane damage by terminating free radical–induced chain reactions.[3] Upon termination of peroxidation reactions, the oxidized tocopheroxy radical produced is then transformed to the reduced state through interactions with other cellular antioxidants, particularly vitamin C.

Vitamin E has additional nonantioxidant functions, including an antiproliferative effect on vascular smooth muscle and an inhibitory influence on platelet aggregation and adhesion.[3] Vitamin E analogues also modulate signal transduction by altering the activity of lipoxygenases, cyclooxygenases, and protein kinase C, and alter gene expression by inhibiting the activation of NF-$\kappa\beta$.[3] Vitamin E additionally suppresses activation of inflammatory cells and protects against Kupffer and stellate cell activation.[1,2]

Literature review

Vitamin E administration has been evaluated in in vitro and animal models of hepatotoxicity. Vitamin E ameliorated oxidant-induced damage in rat hepatocytes exposed to hydrophobic bile acids in vitro, improved liver histology in ethanol-induced liver disease, prevented the development of hepatic steatosis, and reduced mortality associated with carbon tetrachloride toxicity.[12,88,89] In human patients with hepatobiliary disease, results of clinical trials with vitamin E supplementation have been mixed. A few studies have demonstrated biochemical improvement in patients with NASH treated with vitamin E, with some showing improvement in histologic parameters of inflammation and fibrosis.[90,91] In one small clinical trial, a combination of vitamin E and UDCA improved laboratory values and hepatic steatosis scores in patients with NASH better than UDCA therapy alone.[92] The results in alcoholic liver disease and viral hepatitis have been less impressive, although one small pilot study of vitamin E combined with antiviral therapy resulted in a 2.4 times greater chance of a complete response when compared with antiviral therapy alone.[93]

The effects of vitamin E supplementation in veterinary patients with hepatobiliary disease have not been reported except for a small pilot study of 20 dogs with chronic hepatitis fed a vitamin E–supplemented diet for 3 months. In these dogs, increases in serum and hepatic vitamin E concentrations were accompanied by an increased hepatic GSH:GSSG ratio, suggestive of an improved hepatic redox status, but no changes in clinical or histologic scores were noted.[94]

Dose and pharmacokinetics

The recommended oral formulation of vitamin E is the acetate form of α-tocopherol.[5] It is commercially available as d-α–tocopherol, a synthetic form of vitamin E, comprised of the eight possible stereoisomers in equal amounts. A dose of 10 to 15 IU/kg/d is recommended for dogs and cats that have necroinflammatory or cholestatic liver disorders. Higher doses may be indicated in animals with severe cholestatic disorders that compromise fat absorption. An emulsified formulation is available (Vedco, Agri-Labs, St. Joseph, Missouri; Durvet, Blue Springs, Missouri; Schering-Plough,

Kenilworth, New Jersey) for parenteral administration and should be considered in animals with severe cholestasis. In a recent study in a rodent model, emulsified vitamin E ameliorated acute hepatobiliary injury induced by administration of hydrophobic bile acids.[88] This formula may prove to be useful in the management of acute cholestatic hepatopathies such as idiopathic hepatic lipidosis in cats.

Side effects

Vitamin E is generally considered to be of low toxicity. In humans, high doses (>5000 IU/d) can antagonize the absorption of other fat-soluble vitamins, resulting in impaired bone mineralization, reduction in hepatic vitamin A stores, and coagulopathy secondary to vitamin K insufficiency.[5]

Use in veterinary medicine

Vitamin E supplementation should be considered in the management of hepatobiliary disorders likely to involve oxidative membrane injury, such as cholestatic hepatopathies (hepatic lipidosis in cats), specific hepatotoxins, necroinflammatory hepatobiliary disease, ischemia-reperfusion injury, and transition metal toxicity (copper and iron).

SUMMARY

Although studies in in vitro systems and animal models provide compelling data supporting the hepatoprotective benefits of the cytoprotective agents discussed in this article, clinical studies in naturally occurring hepatobiliary disease have not been as convincing. In many cases, especially in veterinary medicine, this problem is due to the lack of well-designed, randomized, controlled trials evaluating the efficacy of these agents. In vitro studies and animal models suggest that these agents augment and enhance natural hepatic defense mechanisms to inhibit inflammation and fibrosis, prevent apoptosis, and protect against oxidant injury; however, these agents do not address the primary cause of liver injury, and their utility in hepatobiliary disease is of an ancillary nature. The importance of obtaining a definitive diagnosis via liver histopathology, cultures of liver and bile, and the use of special histopathologic stains cannot be overemphasized. A favorable prognosis is most likely when a definitive diagnosis is obtained early in the course of the disease with treatment directed at the underlying cause. Once a definitive diagnosis is obtained and the ongoing pathology is understood, the clinician will be able to select the most appropriate cytoprotective agent. Ideally, in the future, we will endeavor to pursue clinical trials of these cytoprotective agents in our patients to provide a more justified rationale for their use.

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