Milk Thistle and Its Derivative Compounds: A Review of Opportunities for Treatment of Liver Disease

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Milk thistle extracts have been used as a "liver tonic" for centuries. In recent years, silibinin, the active ingredient in milk thistle extracts, has been studied both in vitro and in vivo to evaluate the beneficial effects in hepatic disease. Silibinin increases antioxidant concentrations and improves outcomes in hepatic diseases resulting from oxidant injury. Silibinin treatment has been associated with protection against hepatic toxins, and also has resulted in decreased hepatic inflammation and fibrosis. Limited information currently is available regarding silibinin use in veterinary medicine. Future study is justified to evaluate dose, kinetics, and treatment effects in domestic animals.

Key words: Antioxidant; Hepatic; Silibinin; Silymarin.

Silybum marianum, or milk thistle, frequently is utilized in the treatment of humans and animals with hepatic failure. Up to one-third of people reported using milk thistle compounds to supplement therapy for chronic viral hepatitis,[1] and other clinic-based reports suggest that use by persons with chronic liver disease is even higher.[2,3] Milk thistle seed extracts have been used for centuries for liver protection.[4,5] Worldwide, researchers continue to show interest in milk thistle derivatives as a potential treatment for several diseases as evidenced by the over 12,000 related scientific publications produced on this subject within the last 10 years.[6] This review is confined primarily to the liver protective effects of milk thistle derivatives.

Nomenclature

Milk thistle derivative nomenclature can be confusing because of composition of similar named compounds and identical compounds with alternate names.[7] Silymarin is a standardized extract of milk thistle fruits and seeds containing at least 7 flavonolignans (including silibinin, isosilibinin, silychristin, isosilychristin, and silydianin) and 1 flavonoid (taxifolin).[8] Silibinin is the predominant compound in silymarin, composing 50–70%.[6] Silibinin, also referred to as silybin, exists as a 1 : 1 mixture of 2 diastereoisomeric compounds, silybin A and silybin B. Isosilibinin similarly is a diastereoisomeric compound. Silibinin is not only the predominant ingredient in silymarin, but it also is considered to be the primary active ingredient.[9] For this reason, compounds containing milk thistle

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ingredients report silibinin content. Milk thistle-derived formulations for IV use, such as those licensed for *Amanita* intoxication, are composed only of the diastereoisomeric silibinin flavonolignans.[10]

Pharmacokinetics

Milk thistle extracts have low bioavailability when administered PO in most species and, after absorption, are primarily conjugated by glucuronidation and excreted into bile and urine with minimal phase I metabolism.[4] Silibinin has been shown to be glucuronidated at 3 phenolic OH groups at positions 28, 31, and 32 (Fig 1) in vitro by ovine liver glucuronyl transferase.[11] Species and stereoisomer specific glucuronidation patterns have been observed, but these 3 silibinin glucuronides have been shown to be enzymatically generated in biological systems. Extensive conjugation and biliary excretion result in short half-lives and low systemic exposure.[12] Its primary phase II metabolite, silibinin glucuronide, is transported by biliary flow to the intestinal tract where it undergoes cleavage by bacterial β-glucuronidase enzymes, restoring the silibinin parent compound and promoting enterohepatic circulation. Because of the route of metabolism and excretion, bile silibinin concentrations are approximately $100\times$ higher than serum concentrations.[13] Minimal in vitro inhibition of CYP3A4 and CYP2C9 has not translated into significant in vivo decreases in specific drug metabolism, and clinical interactions have not been reported.[14–16] Despite this lack of interaction, many authors prudently suggest caution in administering high doses of silibinin to ill patients receiving several medications.[17]

Silibinin is relatively insoluble in water and is not absorbed readily from the intestines.[18] Bioavailability can be improved by combining milk thistle extracts with solubilizing substances. Complexing with
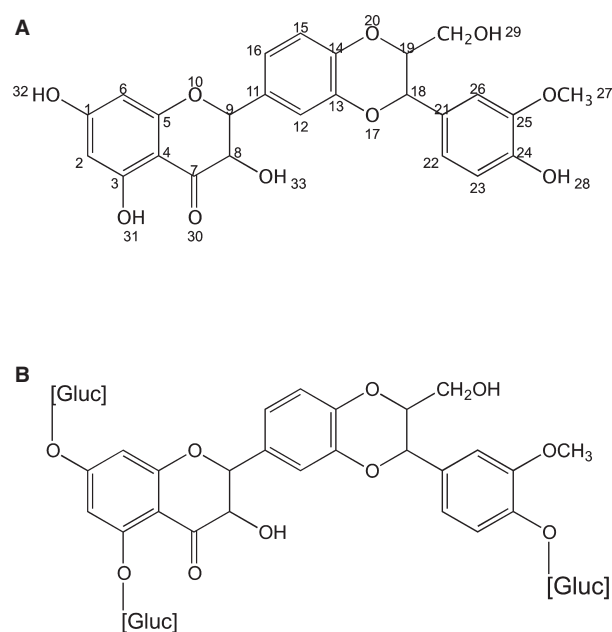
A

B

Fig 1. Chemical structure of the flavonolignan silibinin (A) and its primary phase II metabolite silibinin glucuronide (B).

phosphatidylcholine increases oral bioavailability of silibinin in healthy humans and in patients with hepatic cirrhosis.[5,19] Measurement of systemic plasma concentrations allows estimation of gastrointestinal drug absorption. Commercially available milk thistle products are highly variable in content, dissolution, and bioavailability, making it important to perform pharmacokinetic testing of products in the species of interest before clinical use.[20] Silibinin does not accumulate in plasma with an 8-hour chronic dosing interval in people with noncirrhotic chronic hepatitis.[12] Drug exposure is increased 3- to 5-fold in people with hepatic disease, with the highest concentrations seen in nonalcoholic fatty liver disease and cirrhosis.[21] Increases in drug exposure caused by liver disease may be related to decreased phase II conjugation and transporter proteins.[21] Although silibinin exhibits linear pharmacokinetics when administered to people with normal hepatic function, nonlinear pharmacokinetics have been reported when higher dosages are given to patients with hepatitis.[12,22,23] Enterohepatic circulation, and resultant hepatic and intestinal silibinin concentrations, may contribute to this phenomenon. Nonlinear pharmacokinetics observed at higher dosages in people with liver disease could, in part, counteract low bioavailability and improve therapeutic results in this target population.

Pharmacokinetic studies performed in dogs mirror studies in humans demonstrating improved bioavailability of silibinin complexed with phosphatidylcholine over standardized milk thistle extracts.[24] Preparation of silymarin in fluid-bed coated pellets and silibinin in sodium cholate/phospholipid-mixed micelles resulted in similar increased silibinin absorption in dogs.[25,26] Differences in feline hepatic metabolism characteristics

have made it difficult to extrapolate pharmacokinetics between cats and other species. Therefore, pharmacokinetics of silibinin complexed with phosphatidylcholine have been established recently in cats.[27] Oral bioavailability is enhanced to approximately 7% in cats due in part to limited glucuronidation capacity in this species. Pharmacokinetic behavior of silibinin remains largely unknown in large animal herbivores. Although plasma pharmacokinetic analyses have not been reported in dairy cattle, silibinin has not been detected in milk from cows drenched with milk thistle extract.[28] Silibinin complexed with phosphatidylcholine, manufactured wholesale,[a] is available to animal health providers from multiple retail sources.[b,c]

Pharmacodynamics

Although milk thistle extracts have been used for centuries as a liver tonic, only recently has the mechanism of hepatic protection become better understood.[4] Silibinin is a drug with multiple functions and targets (Table 1). The mechanism of action best known is antioxidant free radical scavenging and inhibition of lipid peroxidation[29]. Hepatic injury has long been linked to oxidative injury.[30] Silibinin is most effective in scavenging low molecular weight free radicals, such as the hydroxyl radical.[31] Silibinin has been shown to be protective against oxidant injury in vitro in peripheral blood,[32,33] hepatocytes,[34] and in several other body tissues.[25] In vivo studies indicate that silibinin is protective against oxidant injury in several body tissues.[35,36] Rats supplemented with silibinin showed a tissue-specific increase in glutathione content, primarily in hepatic and intestinal tissues.[37] Investigators also have documented improvement in feline granulocyte GSH content after 5 days of silibinin administration.[38]

Table 1. Summary of silibinin cellular effects relevant to treatment of liver disease.

Silibinin Cellular Effects
Free radical scavenging
Inhibition of lipid peroxidation
Suppression of NF-κB translocation and binding
Suppression of TNFα, TNF receptor 1, and TNF receptor 1 – associated apoptosis-ligand expression
Decreased IL-4 expression
Inhibition of 5-lipooxygenase pathway and leukotriene formation in Kupffer cells
Inhibition of nitric-oxide synthase expression secondary to LPS stimulation
Reduced monocyte chemoattractant protein 1 with IL-1β stimulation
Reduced IL-1β and and prostaglandin E2 in LPS sepsis
Inhibition of selectin adhesion molecule expression
Reduction in stellate cell DNA synthesis, proliferation, and migration
Reduction in hepatic collagen, procollagen III, procollagen α1, and profibrogenic mRNA expression
Up-regulation of bile salt export pump and choleresis
Competitive inhibition of hepatocyte-specific OATP2 transporters

Silibinin treated cells are more resistant to cell lysis upon exposure to oxidizing agents.[5]

The anti-inflammatory effects of silibinin extend beyond inhibition of reactive oxygen species-dependent mechanisms. Many of the anti-inflammatory properties of silibinin are caused by nuclear DNA/RNA-mediated effects, via suppression of NF-κB translocation and binding.[39] Silibinin suppressed tumor necrosis factor α (TNF), TNF receptor 1 and TNF receptor 1-associated apoptosis-ligand expression, as well as associated hepatic apoptosis and increases in liver enzyme activity in mice treated with fumonisin.[40] Silibinin also has been shown to significantly inhibit TNF and interleukin 4 expression in a model of acute hepatitis in mice.[41] Silibinin strongly inhibits the 5-lipooxygenase pathway and leukotriene formation in Kupffer cells in vitro.[42] Secondary to lipopolysaccharide (LPS) stimulation, silibinin inhibits inducible nitric-oxide synthase expression in vitro.[43] Multiple in vitro studies in stellate cells and hepatocytes have demonstrated a reduction in monocyte chemoattractant protein 1 secondary to interleukin (IL) 1β stimulation.[44–46] IL-1β and prostaglandin E2 have been decreased in vivo with silibinin administration, which also improved survival in a mouse LPS sepsis model.[47] In addition, recent evidence suggests that silibinin inhibits selectin adhesion molecule expression, necessary for leukocyte migration.[48]

Silibinin has been shown to have an antifibrotic effect in the liver. Central to the onset of hepatic fibrosis is conversion of hepatic stellate cells to myofibroblasts, which is limited by silibinin by interruption of cell signaling. Silibinin decreases stellate cell DNA synthesis, proliferation, and migration.[44] Silibinin also limits fibrous tissue production. In a hepatic injury model in rats, silibinin administered PO decreased liver collagen concentration up to 55%.[49] Silibinin also decreased collagen, procollagen III, procollagen α1, and profibrogenic mRNA expression by 30% in rats with biliary obstruction.[50,51] In a study in which ethanol and silibinin were administered over a period of 3 years to baboons, concentrations of hepatic collagen type I, procollagen mRNA, and the incidence of alcohol-induced hepatic fibrosis and cirrhosis were found to be lower than in the control group.[52] Milk thistle extracts decreased markers of inflammation and fibrosis when used in combination with praziquantel an in vivo model of schistosomal liver fibrosis.[53]

Hepatoprotective effects of silibinin in liver disease may be related to mechanisms of enhanced protein synthesis. In an in vivo study, silibinin differentially increased DNA synthesis in partially hepatectomized rats, but not in rats in the healthy control group or in those with hepatic neoplasia.[54] Protein synthesis is necessary for hepatic regeneration and repair after toxic and inflammatory insults. Another hepatoprotective effect of silibinin is prevention of estrogen- and taurolithocholate-induced cholestasis by up-regulation of the bile salt export pump.[18] Bile transport across the canalicular membrane is the rate-limiting step in bile flow, and silibinin may be beneficial in some forms of acquired hepatocellular cholestasis.[55] Silibi-

nin administration resulted in a dose-dependent increase in bile flow (choleresis), primarily because of stimulation of the rate of bile salt synthesis in vivo in rats.[56]

Safety

Several clinical trials in humans have been performed to evaluate the toxicity and efficacy of silibinin treatment for hepatic disease.[57] Studies demonstrated that toxicity is low in humans and animals, adding to the reputation of silibinin treatment as safe and nonharmful. In a 2-year toxicity study in healthy rats and mice, animals consuming silibinin had a significantly lower incidence of hepatic mixed inflammatory cell infiltration, bile duct hyperplasia, and spontaneous hepatocellular adenoma and carcinoma.[58] The predominant adverse events reported in people consuming silibinin are headaches and pruritus, with a lesser incidence of diarrhea and nausea.[59] Total percentage of adverse events reported in a meta-analysis of clinical trials was 2.36% with silibinin versus 5.05% with placebo.[59] No deaths or life-threatening adverse events have been reported.[59] Laxative effects of silibinin at higher doses may be related to carrier elements or increased bile secretion.[22] This implies that increases in dosage may, in turn, increase risk of adverse events. Higher dosage studies have not been performed in healthy people; therefore, associated adverse events may be related to primary disease conditions.[60]

Therapeutic Applications

Intoxication

Convincing evidence exists to support silibinin treatment for hepatic toxins. Silibinin treatment decreases the mortality rate nearly 50% in people suffering from *Amanita phalloides* intoxication compared with those untreated with silibinin.[59] Silibinin also is protective for *Amanita* intoxication when administered experimentally in dogs.[61,62] In dogs, silibinin limits changes in biochemical and coagulation parameters, decreases degree of hepatic hemorrhagic necrosis, and prevents death.[61,62] In addition to limiting oxidation, silibinin prevents transport of the phalloidin toxin into hepatocytes by competitive inhibition of hepatocyte-specific OATP2 transporters.[5] Silibinin also inhibits hepatocyte uptake of other toxins by membrane bile salt-binding polypeptides.[18] In 2 reports, silibinin treatment improved hepatic function in workers with environmental exposure to hepatotoxic industrial solvents.[63,64] Silibinin treatment has been shown to prevent increases in hepatic enzyme activity and other toxic changes in rats treated with carbon tetrachloride,[65,66] acetaminophen,[67] and arsenic.[68] Silibinin also prevented iron-induced hepatotoxicity in rats. Prophylactic use of silibinin may protect against iatrogenic toxins as well. In a study evaluating silibinin treatment combined with a hepatotoxic antituberculosis drug, hepatic enzyme activity was significantly decreased.[5]

Silibinin also has been shown to be protective against radiation-induced hepatic injury and increases in hepatic enzyme activity, as well as that induced by doxorubicin.[69–71]

Hepatitis

Silibinin currently is recommended for use in alcoholic liver disease. Ethanol induces free radical formation through multiple pathways, resulting in steatohepatitis and cirrhosis with chronic use.[59] Patients with alcoholic liver disease undergoing 6 months of treatment with silibinin had improved cellular superoxide dismutase activity and serum glutathione peroxidase activity.[72] In 1 in vitro study performed using human hepatocytes, silibinin completely prevented ethanol-induced release of lactate dehydrogenase.[73] Double-blinded placebo-controlled studies have demonstrated significant decreases or normalization of hepatic enzyme activity in alcoholic liver disease, as well as improvement in symptoms of anorexia, nausea, and asthenia.[59] Improvement in histological scores of hepatic pathology have also been observed in these patients.[74] A recent Cochrane meta-analysis concluded that although milk thistle derivatives could affect outcomes in patients with alcoholic liver disease, additional randomized clinical trials are needed.[75]

Silibinin commonly is recommended for use in viral hepatitis. Although silibinin has no known direct suppressive effects on viral replication, its use targets inhibition of inflammation and cytotoxic events secondary to viral infection.[59] In patients with chronic hepatitis, silibinin decreases transaminase activity caused by hepatic damage and decreases serum malondialdehyde concentrations, a marker of oxidative injury.[6,76] Silibinin is well tolerated in human subjects with chronic hepatitis C infection.[77] Use of silibinin is accompanied by better quality of life and fewer symptoms in these patients.[1,78]

Nonalcoholic fatty liver disease occurs because of metabolic stress or can accompany primary hepatitis, promoting progression of inflammatory disease to fibrosis.[6] In in vivo models of nonalcoholic fatty liver disease, silibinin supplementation limits hepatic glutathione depletion and hydrogen peroxide production, prevents hepatic mitochondrial dysfunction, decreases hepatic enzyme activities, and improves liver histology.[79–82] Silibinin use has undergone clinical trials in this population of human patients. Recent studies demonstrate improvement in liver enzyme activities and liver histology in patients with nonalcoholic fatty liver disease after silibinin treatment.[83,84] Silibinin also decreases C-reactive protein, inflammatory cytokines, indices of hepatic fibrosis, and degree of steatosis evaluated ultrasonographically in this population.[83,85,86] When silibinin was administered to peripartum dairy cows (in which subclinical fatty liver disease is common), effects included improved lactation performance and improved body condition.[28] Although hepatic biopsies confirmed fatty liver changes in both silibinin treated and control groups, distribution of histopathologic changes within the lobule differed.[87] In silibinin treated cows, only hepatocytes nearest the central vein were consistently affected, whereas in control cows, hepatocytes throughout the lobule contained cytoplasmic vacuoles.[87]

Cirrhosis

Hepatic cirrhosis is a common end result of advanced stages of alcoholic liver disease and viral hepatitis. Remodeling of hepatic architecture secondary to fibrosis can result in hepatic insufficiency, portal hypertension, and hepatic encephalopathy.[5] After the onset of cirrhosis, treatments targeted at the underlying cause are of limited efficacy.[59] Use of silibinin in hepatic cirrhosis results in improvement in antioxidant status, cytoprotection, reversal of fibrosis, and regeneration. Dose-dependent decreases in hepatic enzyme activity with silibinin treatment have been documented.[88] In a placebo-controlled trial, patients with cirrhosis consuming silibinin had greater total glutathione concentrations and concurrent decreases in N-terminal propeptide of type III collagen, a biomarker for hepatic fibrosis.[89] Decreased mortality rates have been documented with silibinin use in randomized controlled trials performed in patients with cirrhosis.[90] All-cause mortality decreased 4.4% and mortality from liver causes decreased 7.3% in cirrhotic patients.[59]

A meta-analysis of clinical trials that reviewed the evidence for silibinin use in people with liver disease specifically addressed liver-related mortality. Liver-related mortality was evaluated over all studies and found to be 10% with silibinin versus 17.3% with placebo.[59] This reduction in liver-related mortality was significant ($P = .01$).[59] In addition, fewer patients were hospitalized for cirrhosis-related issues, with 10.0% hospitalized with silibinin use versus 16.9% hospitalized with placebo use ($P = .086$).[59] The incidence of complicating conditions such as hepatocellular carcinoma, upper gastrointestinal bleeding, and diabetes mellitus also was lower in silibinin treated patients.[59,90]

Randomized clinical trials have not been performed in clinically affected animals with hepatic diseases. Studies evaluating silibinin administration thus far have only been performed in dogs, cats, and dairy cattle. However, much can be learned from preliminary studies in animal models and adapted from these and clinical studies in humans. Despite evidence for use, silibinin is not considered a sole treatment, but an adjunctive drug indicated in a variety of acute and chronic diseases affecting liver function. Limited information is available regarding silibinin disposition and pharmacodynamics in both domestic small animals and large animal herbivores. Additional pharmacokinetic and pharmacodynamics studies in healthy animals, utilizing an agent with potential therapeutic potential in disease, are a necessary first step before evidence-based clinical application.

Footnotes

Acknowledgment

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