DMD Fast Forward. Published on June 19, 2008 as doi:10.1124/dmd.107.019604

DMD#19604

Title Page

Title: The Pharmacokinetics of Silymarin is Altered in Patients with Hepatitis C Virus and Nonalcoholic Fatty Liver Disease and Correlates with Plasma Caspase-3/7 Activity

Authors: Sarah J. Schrieber, Zhiming Wen, Manoli Vourvahis, Philip C. Smith, Michael W. Fried, Angela D. M. Kashuba, and Roy L. Hawke.

Division of Pharmacotherapy & Experimental Therapeutics, UNC Eshelman School of Pharmacy (SJS, ZW, MV, PCS, ADMK, RLH); and Division of Gastroenterology and Hepatology, School of Medicine (MWF), University of North Carolina, Chapel Hill, NC, USA.

Running Title Page

Running Title: Pharmacokinetics of Silymarin is Altered in Liver Disease Patients

Corresponding Author: Roy L. Hawke, Pharm.D., Ph.D.

Division of Pharmacotherapy and Experimental Therapeutics

UNC Eshelman School of Pharmacy,

CB #7360, Kerr Hall Rm 3310

Chapel Hill, NC 27599-7360

Fax: 919-962-0644

Email: rhawke@email.unc.edu

Document

Text pages ……	17
Tables ……	3
Figures ……	4
References ……	40
Words in Abstract ……	250
Words in Introduction …	720
Words in Discussion ……	1286

ABBREVIATIONS

HCV, hepatitis C virus; SC, silychristin; SD, silydianin; SA, silybin A; SB, silybin B; ISA, isosilybin A; ISB, isosilybin B; LC-MS, liquid chromatography-mass spectrometry; HPLC, high performance liquid chromatography; C_{max}, maximum plasma concentration; T_{max}, peak time at C_{max}; $t_{1/2}$, terminal elimination half-life; *CL/F*, apparent clearance; $AUC_{0\rightarrow 24h}$, area under the plasma concentration-time curve from time 0 to 24 hours; ALT, alanine aminotransaminase

DMD Fast Forward. Published on June 19, 2008 as DOI: 10.1124/dmd.107.019604
This article has not been copyedited and formatted. The final version may differ from this version.

ABSTRACT *Background/Aims:* Silymarin, used by 30 - 40% of liver disease patients, is comprised of 6 major flavonolignans each of which may contribute to silymarin's hepatoprotective properties. Previous studies have only described the pharmacokinetics for two flavonolignans, silybin A and silybin B, in healthy volunteers. The aim of this study was to determine the pharmacokinetics of the major silymarin flavonolignans in liver disease patients. *Methods*: Healthy volunteers and three patient cohorts were administered a single, 600 mg oral dose of milk thistle extract and fourteen blood samples were obtained over 24 hours. *Results:* Silybin A and B accounted for 43% of the exposure to the sum of total silymarin flavonolignans in healthy volunteers and only 31 - 38% in liver disease cohorts due to accumulation of silychristin (20 - 36%). $AUC_{0\text{-}24h}$ for the sum of total silymarin flavonolignans were 2.4-, 3.3-, and 4.7-fold higher for hepatitis C virus (HCV) noncirrhosis, nonalcoholic fatty liver disease ($p \leq 0.03$), and HCV cirrhosis cohorts ($p \leq 0.03$), respectively, compared to healthy volunteers ($AUC_{0\text{-}24h}$=2021 ng*h/ml). Caspase-3/7 activity correlated with the $AUC_{0\text{-}24h}$ for the sum of all silymarin conjugates among all subjects (R^2=0.52), and was 5-fold higher in HCV cirrhosis cohort ($p \leq 0.005$ vs healthy). No correlation was observed with other measures of disease activity including plasma ALT, IL-6, and 8-isoprostane $F_{2\alpha}$, a measure of oxidative stress. *Conclusions:* These findings suggest that the pharmacokinetics of silymarin is altered in patients with liver disease. Patients with cirrhosis had the highest plasma caspase-3/7 activity and also achieved the highest exposures for the major silymarin flavonolignans.

DMD Fast Forward. Published on June 19, 2008 as DOI: 10.1124/dmd.107.019604
This article has not been copyedited and formatted. The final version may differ from this version.

Silymarin, an extract of milk thistle (*Silybum marianum*), is an herbal medicine that has been used for centuries to self-treat liver disease. High public perception of silymarin's therapeutic benefits is suggested from the use of this complementary alternative medicine by 30-40% of patients with liver disease (Russo et al., 2001). Silymarin is comprised of six major flavonolignans: silybin A; silybin B; isosilybin A; isosilybin B; silychristin; and silydianin. Antioxidant (Psotova et al., 2002; Kren et al., 2000), anti-inflammatory/immunomodulatory (Manna et al., 1999; Schumann et al., 2003), and anti-fibrotic (Crocenzi et al., 2006) properties of silymarin have been demonstrated in various *in vitro* and animal models. Whether one or more of these six silymarin flavonolignans are responsible for potentially hepatoprotective effects in patients with liver disease is unknown.

Oxidative stress, inflammation, and fibrosis are characteristics of chronic liver disease, and provide the rationale for investigations on the effect of silymarin on disease progression in the absence of direct antiviral activity. Although silymarin appears to be well tolerated, the therapeutic benefits of silymarin have not been consistently demonstrated in various liver disease populations (Saller et al., 2001; Jacobs et al., 2002; Rambaldi et al., 2007; Mayer et al., 2005). For example, changes in standard surrogate clinical endpoints, such as serum alanine aminotransaminase (ALT), have not been observed in patients with chronic hepatitis C and early stage of disease (Buzzelli et al., 1994; Par et al., 2000; Tanamly et al., 2004; Gordon et al., 2006). Other studies suggest silymarin may have anti-fibrotic activity and may decrease the complications of liver disease and mortality in patients with cirrhotic disease (Ferenci et al., 1989; Pares et al., 1998; Lucena et al., 2002). In addition, silymarin has been shown to reduce insulin resistance and lipid peroxidation in cirrhotic diabetic patients (Velussi et al., 1997) which are metabolic complications also observed in patients with non-alcoholic steatohepatitis.

However, the use of different silymarin regimens and lack of information on the silymarin exposures attained in these various patient populations make it difficult to draw conclusions on the efficacy of silymarin and on which patient population should be targeted for further clinical investigation.

The pharmacokinetics for only two of the six major silymarin flavonolignans, silybin A and silybin B, have been extensively studied since they are also contained in two phospholipid formulations with better bioavailability, silipide and silybin-phytosome. However, neither of these formulations has been studied in patients with liver disease, while the pharmacokinetics of silymarin has only been described in healthy volunteers (Weyhenmeyer et al., 1992; Rickling et al., 1995; Wen et al., 2008). Extensive first-pass phase 2 metabolism presumably accounts for the low systemic exposures that have been observed with customary doses of silymarin. For example, plasma AUCs for total flavonolignans (which reflect parent plus conjugated flavonolignans) have been reported to be 3- to 4-fold and 12- to 36-fold higher for silybin A and silybin B, respectively, compared to AUCs for parent flavonolignans (Weyhenmeyer et al., 1992; Rickling et al., 1995; Wen et al., 2008). Silymarin conjugates likely undergo primarily biliary excretion since only about 5% of the dose is recovered as conjugates in urine (Lorenz et al., 1984; Weyhenmeyer et al., 1992). Silymarin's disposition may be altered in liver disease since some phase 2 conjugation pathways and transporter proteins that could be involved in the active transport of flavonolignans have been shown to be decreased in patients with liver disease (Congiu et al., 2002; Guardigli et al., 2005; Hinoshita et al., 2001). Thus, differences in silymarin's pharmacokinetics and systemic exposures may account for inconsistencies in clinical outcomes that have been observed between patients with mild and cirrhotic liver disease.

To determine if silymarin's disposition is influenced by the severity or type of liver disease, we conducted a single dose pharmacokinetic study with a standardized milk thistle extract in three patient cohorts that differed by stage and type of liver disease. A healthy volunteer cohort was also included for comparison to patient cohorts and for reference to previous investigations. The pharmacokinetics of six major silymarin flavonolignans and their conjugates were determined and correlated with ALT and with 8-isoprostane $F_{2\alpha}$ and caspase-3/7 activity, as plasma measures of oxidative stress and apoptosis, respectively. Sulfate and glucuronide conjugate pools for the major silymarin flavonolignans were also examined to gain additional insight on how liver disease might influence silymarin's metabolism and disposition.

DMD Fast Forward. Published on June 19, 2008 as DOI: 10.1124/dmd.107.019604
This article has not been copyedited and formatted. The final version may differ from this version.

Materials and Methods

Subjects and Study Design. This single-dose, open-label, non-randomized study enrolled 5 subjects into each of the four cohorts (n=20). The primary objective of this study was to determine preliminary point estimates and variance information for the pharmacokinetic parameters AUC, C_{max}, t_{max}, CL/F, and $t_{1/2}$ in healthy volunteers and in patients diagnosed with either HCV and minimal liver disease or cirrhosis, or nonalcoholic fatty liver disease (NAFLD) receiving a single 600 mg dose of milk thistle extract. Assuming an absence of disease effects, the selected sample size of N = 5 per each cohort was based on historical experience in healthy subjects and not on statistical considerations. However, an *a priori* power calculation suggested that a two-sided t-test would have 90% power to detect a 2.5-fold increase in the AUC for total silybin A + silybin B at an α=0.05 using previously reported mean and variance data from healthy volunteers (Weyhenmeyer et al. 1992).

Male and female subjects aged 18 to 65 years with a body weight ≥ 50 kg were eligible without regard to smoking status. A healthy volunteer cohort was identified by medical history, screening physical examination, vital signs, and clinical laboratory measurements. Two chronic HCV patient cohorts consisted of non-responders to interferon-based therapies; one cohort without cirrhosis (Metavir stage I or II) and the other with cirrhosis (Metavir stage III or IV). The final patient cohort consisted of NAFLD patients confirmed by a diagnostic biopsy within six months of study participation or by serologies that confirmed the exclusion of other liver diseases. Exclusion criteria included: pregnant or lactating females; other active liver diseases; HIV co-infection; history of pancreatic or biliary disease; acute illness that would interfere with drug absorption; allergy or hypersensitivity reaction to milk thistle or any of its components; use of any silymarin-containing product within 30 days prior to enrollment; or use of alcohol within

48 hours of enrollment. Concomitant use of oral contraceptives or inhibitors or inducers of cytochrome P450 3A4 or 2C9 were also excluded due to theoretical concerns for potential drug interactions (Beckmann-Knopp et al., 2000).

Subjects were fasted overnight for 8 to 12 hours and then received a single, 480 mg oral dose of silymarin administered as two 300 mg milk thistle capsules with approximately 240 ml of water. A low-fat research breakfast, lunch, and dinner was served immediately after each dose. Meals were served between 8:30-9:00 am, 12:00-1:30 pm, and 5:00-7:00 pm. Fourteen serial blood samples were collected at time points 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, and 24 hours after silymarin dosing.

The study was conducted at the Verne S. Caviness General Clinical Research Center at the University of North Carolina at Chapel Hill. The study protocol and subject-informed consent were approved by The University of North Carolina institutional review board, and the study was conducted according to the Declaration of Helsinki. All subjects provided written informed consent before enrollment.

Silymarin Dose. A common, commercially available milk thistle extract used by many patients seen at the University of North Carolina Hepatitis Clinic (Nutraceutical Sciences Institute® (NSI), Boynton Beach, FL) was selected for investigation. According to manufacturer's labeling, each capsule contained 300 mg milk thistle extract prepared from seed and was standardized as 80% (240 mg) silymarin. All doses were administered from Lot No. 0418901. The specific flavonolignan content of this milk thistle extract has been previously determined by our laboratory (Wen et al., 2008) as follows: 37.7 mg, silybin A; 58.8 mg, silybin B; 14.8 mg, isosilybin A; 6.3 mg, isosilybin B; 39.2 mg, silychristin; and 15.3 mg, silydianin. Therefore,

these six flavonolignans account for 172 mg, or 57%, of the 300 mg milk thistle extract contained in each capsule (Wen et al., 2008).

Silymarin Flavonolignan Plasma Concentrations. Whole blood samples were collected in two 3 ml EDTA-lined tubes (K_2-EDTA tubes; BD, Franklin Lakes, NJ, USA) and centrifuged at 2400 rpm for 10 minutes at 4^{o}C. The plasma was collected, frozen, and stored at -20^{o}C until analysis. Plasma concentrations of the six silymarin flavonolignans were quantified using a recently described LC-MS method (Wen et al., 2008). Briefly, 100 μl aliquots of the plasma samples were used to determine parent (i.e. nonconjugated) or total (i.e. parent + conjugates) flavonolignan concentrations after a 6 hour incubation at 37^o C, in the absence or presence of a mixture of sulfatase (80 U/ml) and β-glucuronidase (8000 U/ml) (Sigma-Aldrich, St. Louis, MO), respectively. Plasma concentrations of flavonolignan conjugate were estimated by taking the difference in parent flavonolignan plasma concentrations before and after enzymatic hydrolysis with β-glucuronidase and sulfatase. This subtraction method provides an estimate of plasma concentrations of silymarin conjugates expressed in terms of "Parent Flavonolignan Equivalents". Plasma samples were also incubated with either enzyme separately to study the effect of liver disease on silymarin's two pathways of phase 2 metabolism. Concentrations of sulfate and glucuronide conjugates were determined at the T_{max}, 1.5 hours post-dose, for silybin B and isosilybin A (see Figure 3) since they exhibited the highest C_{max} and $AUC_{0\text{-}24h}$ for total flavonolignan concentrations in plasma among the six flavonolignans.

Flavonolignans were separated using an Agilent HP 1050 LC system (Palo Alto, CA) and a Luna C_{18} column (50 × 2.0 mm i.d., 3 μm) and a methanol: 1% acetic acid (44:56 v/v, pH 2.8) mobile phase with isocratic elution at a flow rate of 0.3 ml/min and a run time of 12 minutes.

MS analysis and detection were conducted using an API 100 LC/MS system (PerkinElmer Sciex, Toronto, Canada) with a TurboIonspray interface in the negative ESI ionization mode. The limit of detection and linear quantitative range for the six silymarin flavonolignans were 2-1000 ng/ml and 5-1000 ng/ml, respectively. Intra- and inter-day precisions were 1.7 - 11%, and 4.5 - 14%, respectively. For authentic reference standards, the composition of silybin (Silibinin, Sigma-Aldrich, St. Louis, MO) was confirmed to be a mixture of silybin A (SA) and silybin B (SB) by LC-ESI-MS and the specific contents of SA and SB were analyzed to be 48% and 52%, respectively. Silychristin (SC) was obtained from ChromaDex (Santa Ana, CA), and silydianin (SD) was purchased from U.S. Pharmacopoeia (USP; Rockville, MD). Isosilybin A (ISA) and isosilybin B (ISB) reference standards were obtained as a generous gift from Ulrich Mengs (Madaus GmbH).

Measures of Liver Disease Activity. Plasma IL-6 (Quantikine HS, R&D Systems®, Minneapolis, MN, USA) and 8-isoprostane $F_{2\alpha}$ (Direct ELISA, Assay Designs®, Ann Arbor, MI, USA) concentrations were determined according to manufacturer instructions. Plasma caspase-3/7 activity (Caspase-GLO® 3/7 Assay, Promega®, Madison, WI, USA) was measured using a recently described method by Seidel and colleagues (Seidel et al., 2005) with the following modifications: plasma was diluted 1:10 in buffer and incubated with substrate for 2 hours.

Pharmacokinetic and Statistical Analysis. Pharmacokinetic parameters including: area under plasma concentration-time curve from time 0 to 24 hours ($AUC_{0\text{-}24h}$); maximum plasma concentration (C_{max}); time to C_{max} (t_{max}); apparent clearance (total oral clearance divided by bioavailability, (CL/F)); and terminal half-life ($t_{1/2}$) were calculated for the six parent and total

silymarin flavonolignans for each subject using noncompartmental methods, WinNonlin-Pro (version 4.1; Pharsight Corp, Mountain View, CA, USA). AUC was calculated by the linear up/log down trapezoidal method to the last time point (AUC_{0-24h}). For CL/F calculations, the dose of each silymarin flavonolignan was determined from their specific content in the NSI milk thistle product as described above. All pharmacokinetic parameters are reported as geometric means with their 95% confidence intervals. A one-way ANOVA was conducted on natural log-transformed data using the Dunnett's multiple comparison to test for significant differences between the healthy control group and each of the three different liver disease patient cohorts, $p<0.05$ significant (SAS JMP 6.0.0; SAS Institute, Care, NC, USA).

DMD Fast Forward. Published on June 19, 2008 as DOI: 10.1124/dmd.107.019604
This article has not been copyedited and formatted. The final version may differ from this version.

Results

Subjects. The demographics for study participants are presented in Table 1. The mean age for the three patient cohorts was approximately 20 years older than the healthy cohort. Serum ALT (normal, male: 19 - 72 U/L; female: 12 - 48 U/L) was approximately 2-fold higher for both HCV noncirrhosis and NAFLD cohorts, and approximately 4-fold higher for the HCV cirrhosis cohort compared to the healthy cohort. The HCV cirrhosis cohort was characterized by a lower platelet count (normal 155 - 440 cells/mm^3) while total bilirubin (normal 0 - 1.2 mg/dl) was similar across all cohorts indicative of well-compensated liver disease. Renal function was normal (CrCl >60 ml/min) for all study subjects.

Pharmacokinetics of Parent Silymarin Flavonolignans. The plasma C_{max} and $AUC_{0\text{-}24h}$ for six major silymarin flavonolignans are presented in Table 2. Silybin A and silybin B were the main flavonolignans in the plasma for all cohorts and their C_{max} ranged from 12 ng/ml (healthy) up to 69 ng/ml (HCV cirrhosis), and from 9 ng/ml (healthy) up to 40 ng/ml (NAFLD), respectively. Silybin A and silybin B exposures ($AUC_{0\text{-}24h}$) were also higher in patient cohorts compared to the healthy cohort. C_{max} and $AUC_{0\text{-}24h}$ for the other silymarin flavonolignans (ISA, ISB, SC, and SD) were only consistently quantifiable in the liver disease cohorts. The HCV cirrhosis cohort had the highest $AUC_{0\text{-}24h}$ for all silymarin flavonolignans. Absorption from the gastrointestinal tract was rapid for all cohorts as indicated by a median T_{max} between 0.5 to 2 hours for the various flavonolignans. By 6 hours post-dose, flavonolignan concentrations had fallen below the detection limit in a majority of subjects due to short elimination half-lives (0.6 to 1.6 hours) for the silymarin flavonolignans observed in all cohorts (data not shown).

The apparent clearances for the six major silymarin flavonolignans have not been previously reported and are also presented in Table 2. CL/F's for silybin A and silybin B were between 27 - 48% and 33 - 51% lower, respectively, in the HCV cohorts compared to the healthy cohort. However, these differences were not detected as significant due to large inter-subject variability. CL/F for the NAFLD cohort was comparable to the healthy cohort.

These data suggest that both liver disease etiology and disease stage may be associated with decreases in the clearance of parent silymarin flavonolignans that may result in increased exposures compared to those observed in healthy volunteers.

Pharmacokinetics of Total Silymarin Flavonolignans. Table 3 depicts the plasma C_{max} and $AUC_{0\text{-}24h}$ for the total (parent + conjugates) concentration of each silymarin flavonolignan, which were determined following complete enzymatic hydrolysis of conjugates (sulfates and glucuronides) as described in Materials and Methods. C_{max} and $AUC_{0\text{-}24h}$ for the total concentration of each silymarin flavonolignan were increased by similar extents (1.8- to 6.3-fold and 1.2- to 9.9-fold, respectively) in patient cohorts compared to healthy volunteers. The highest exposures were observed for silybin B, isosilybin A, and silychristin across all disease cohorts, and were highest in the HCV cirrhosis cohort ($p \leq 0.02$). To determine exposures to the total amount of the six silymarin flavonolignans in blood for each cohort, $AUC_{0\text{-}24h}$ for the total concentration of each flavonolignan were summed and evaluated across the four cohorts. $AUC_{0\text{-}24h}$ for the sum of total silymarin flavonolignans were 2.4-, 3.3-, and 4.7-fold higher for the HCV noncirrhosis, NAFLD ($p \leq 0.03$), and HCV cirrhosis ($p \leq 0.03$) cohorts, respectively, compared to healthy volunteers ($AUC_{0\text{-}24h}$=2021 ng*h/ml).

Terminal elimination half-lives for the silymarin flavonolignans ranged from 4 to 10 hours for the healthy cohort compared to 8 to 25 hours in the patient cohorts. The effect of liver disease on the plasma pharmacokinetics for total silymarin flavonolignans is best depicted by comparing the concentration vs time profiles between healthy and HCV cirrhosis cohorts for each silymarin flavonolignan. As seen in Figure 1, the concentration versus time profiles for each of the six total silymarin flavonolignans were elevated in patients with HCV cirrhosis (Panel B) over the 24 hour sampling period compared to those in healthy volunteers (Panel A). Time versus concentration profiles for the HCV noncirrhotic and NALFD cohorts were intermediate to those of the healthy and HCV cirrhosis cohorts and are reflected in the $AUC_{0\text{-}24h}$ data depicted in Table 3.

Pharmacokinetics of Silymarin Flavonolignan Conjugates. To more clearly determine the influence of disease type and severity on the disposition of silymarin conjugates, a "conjugate pool" concentration was calculated at each time point for all cohorts. First, the conjugate (sulfates + glucuronides) concentrations for each silymarin flavonolignan were obtained from the difference between parent (Table 2) and total (Table 3) plasma concentrations. Then the conjugate concentrations for all six silymarin flavonolignans were summed to obtain a "Sum silymarin conjugates" concentration. Figure 2 depicts for each cohort, the concentration versus time profiles for Sum silymarin conjugates expressed in terms of "Parent Flavonolignan Equivalents". These concentration data were used to determine the Sum silymarin conjugates $AUC_{0\text{-}24h}$ which are also depicted in Figure 2 (see Table inset). Compared to healthy volunteers, Sum silymarin conjugates $AUC_{0\text{-}24h}$ were 2.4-, 3.3-, and 4.7-fold greater in HCV noncirrhosis, NAFLD, and HCV cirrhosis cohorts, respectively. The Sum silymarin conjugates C_{max} (data not

shown) and $AUC_{0\text{-}24h}$ were significantly elevated in the NAFLD and HCV cirrhosis cohorts compared to the healthy cohort ($p \leq 0.03$). These increases in Sum silymarin conjugates $AUC_{0\text{-}24h}$ are similar to the increases observed for the sum of total silymarin flavonolignans $AUC_{0\text{-}24h}$ since flavonolignan conjugates account for 97 - 99% of total flavonolignan concentrations. The elimination half-life for patient cohorts ranged from 8 to 10 hours compared to 4 hours for the healthy cohort. Although the 24 hour sampling interval did not allow precise estimates of the terminal elimination phase, these data suggest that the elimination of silymarin conjugates is more prolonged in liver disease.

Silymarin Flavonolignan Metabolism. Figure 3 depicts the relative proportions of glucuronide conjugates for silybin B and isosilybin A, which were between 77 - 86% and 14 - 23%, respectively, across all cohorts. While the primary route of metabolism was glucuronidation for silybin B, and sulfation for isosilybin A, there were no significant effects of disease severity or type on the extent of their conjugation. These data indicate that the higher silymarin flavonolignan conjugate exposures observed in patient cohorts did not reflect alterations in preferred pathways of phase 2 metabolism.

Measures of Disease Activity. To determine if changes in Sum silymarin conjugates $AUC_{0\text{-}24h}$ were associated with various measures of liver disease activity, the Sum silymarin conjugates $AUC_{0\text{-}24h}$ was correlated with measures of oxidative stress and apoptosis in plasma for each of the twenty subjects. As seen in Figure 4, the Sum silymarin conjugates $AUC_{0\text{-}24h}$ correlated with plasma caspase-3/7 activity ($R^2=0.52$, $p<0.001$), a measure of apoptosis. Compared to healthy volunteers, plasma caspase-3/7 activity was 1.3-, 1.1-, and 4.7-fold higher in HCV noncirrhosis,

DMD Fast Forward. Published on June 19, 2008 as DOI: 10.1124/dmd.107.019604
This article has not been copyedited and formatted. The final version may differ from this version.

NAFLD, and HCV cirrhosis ($p \leq 0.005$) cohorts, respectively. In contrast, no correlations were observed with plasma concentrations of either 8-isoprostane $F_{2\alpha}$ (Figure 3 inset), a biomarker of oxidative stress, or between IL-6 or ALT across all patients, and significant differences between cohorts were not detected (data not shown).

Discussion

Single daily doses of silymarin up to 1260 mg per day (Gordon et al., 2006; Huber et al., 2005) have only been studied in patients with early stage liver disease while other trials in patients with advanced disease have utilized three times daily doses of 140 or 150 mg (Ferenci et al., 1989; Pares et al., 1998; Lucena et al., 2002). Previous clinical trials have failed to include measures of silymarin exposure and the inconsistent reports of silymarin's clinical benefits may reflect variation in the exposures attained due to either the effects of liver disease on silymarin's pharmacokinetics, or the different dosing regimens utilized. This is the first investigation of the pharmacokinetics of the six major silymarin flavonolignans and their conjugates in patients with different types and stages of liver disease. Exposures for parent silymarin flavonolignans were generally higher in patient cohorts, especially for isosilybin A, isosilybin B, and silychristin which were not detected in healthy volunteers. However, these exposures were not maintained past six hours post-dose due to low C_{max} concentrations and short half-lives. These data suggest that the silymarin dosing regimens currently used by patients to self-treat their liver disease, or previously evaluated in clinical trials, may not provide adequate plasma exposures to obtain the antioxidant, anti-inflammatory, or anti-fibrotic benefits of silymarin.

Previous silymarin pharmacokinetic studies in healthy subjects have underestimated total silymarin exposures since they have only focused on silybin A and silybin B, which are the major silymarin flavonolignans in milk thistle extracts. Silybin A and silybin B comprised 56% of the flavonolignans in the milk thistle extract used in this study (Wen et al., 2008) but they accounted for only 43% of the sum of total silymarin flavonolignans exposure in healthy volunteers. In patients with liver disease, silybin A and silybin B accounted for even less of the sum of total silymarin flavonolignans (31 - 38%) due the accumulation of silychristin which

accounted for 18% in healthy subjects and 20 - 36% in patients with liver disease. All of the silymarin flavonolignans have been shown to have potent antioxidant activity (Psotova et al., 2002; Kvasnicka et al., 2003), and therefore they may contribute significantly to the clinical effects of silymarin in patients with liver disease.

The increases in peak plasma concentrations and exposures for parent silymarin flavonolignans in patients with liver disease most likely reflect increased intestinal absorption by the gastrointestinal tract and not decreased phase 2 conjugation by either the gut or liver since total silymarin conjugates were also increased. Hepatic and gastrointestinal tissues share many of the same drug transporters that are involved in the absorption of flavonolignans (Morris and Zhang, 2006; Cermak and Wolffran, 2006), and biliary obstruction results in changes in transporter expression in both rat liver and intestine (Kamisako and Ogawa, 2007). Since hepatic expression of many of these drug transporters may be down-regulated in chronic HCV (Hinoshita et al., 2001), the increased absorption of silymarin flavonolignans may reflect a similar down-regulation of transporters within the gastrointestinal tract, such as multidrug resistance protein 2 (MRP2), that might normally limit the absorption of silymarin flavonolignans.

In our study, the most significant alteration in the disposition of silymarin in patients with liver disease was reflected in the plasma concentrations for the sum of total silymarin flavonolignans where exposures were 2.4- to 4.7- fold higher in patient cohorts compared to healthy volunteers. The difference in the mean age between the healthy cohort (30 ± 17 years) and liver disease cohorts (e.g. 53 ± 4 years HCV cirrhosis) may be a limitation to our study because of the potential for age-related differences in metabolism. However, it is unlikely that the 2.4- to 4.7-fold differences in silymarin exposures between liver disease cohorts and healthy

volunteers can be explained by age differences since ages overlap between cohorts and significant influences of age on the disposition of drugs that primarily undergo high first-pass, phase 2 metabolism by the glucuronosyl transferase system have not been observed.

The extent of phase 2 conjugation by either glucuronidation or sulfation pathways for silybin B and isosilybin A was unaffected by liver disease stage or type. Therefore, the elevated plasma levels of phase 2 conjugates of silymarin may result primarily from alterations in hepatic excretion processes rather than from increased phase 2 metabolism. Similar increases in flavonolignan exposures have been reported in a rat model of cirrhosis where an approximately 2-fold increase in plasma AUC for silybin A and B conjugates was correlated with a 50% reduction in the bile to blood exposure ratio for silybin A and B conjugates in cirrhotic rats compared to control (Wu et al., 2008). In humans, decreased biliary excretion of flavonolignan conjugates may potentially influence the efficacy of silymarin due to reduced enterohepatic recycling and return of parent flavonolignans via portal blood. In addition, different types of liver disease or liver injury have been shown to induce different changes in the expression of hepatic transporters in humans (Barnes et al., 2007) and in animal models (Lickteig et al., 2006).

Our data suggest that one measure of liver disease activity, a simple biochemical assay of caspase-3/7 activity in blood, may be useful for predicting the disposition of drugs that undergo extensive conjugation and biliary elimination like silymarin in patients with liver disease. In chronic HCV patients, apoptosis and serum caspase-3/7 activity correlate with liver disease grade and stage (Seidel et al., 2005; Calabrese et al., 2000; Bantel et al., 2001; Bantel et al., 2004). Caspase-3/7 activity reflects the net contributions of several activators of apoptosis because of their downstream location in both the intrinsic and extrinsic pathways of apoptosis. In contrast to caspase-3/7 activity, plasma levels of 8-isoprostane $F_{2\alpha}$, IL-6, and serum ALT

DMD Fast Forward. Published on June 19, 2008 as DOI: 10.1124/dmd.107.019604
This article has not been copyedited and formatted. The final version may differ from this version.

values did not correlate with $AUC_{0\text{-}24h}$ for Sum silymarin conjugates. Altered hepatic expression of biliary transporters was shown to be independent of the inflammation and oxidative stress associated with bile duct-ligation (Wagner et al., 2005). Therefore, other components of disease activity, perhaps related to the development of cirrhosis, may account for the association between caspase-3/7 activity and altered disposition of silymarin conjugates which was most apparent in the HCV cirrhotic cohort. Alternatively, hepatocytes undergoing apoptosis may represent that fraction of the liver with decreased ability to eliminate conjugates of silymarin flavonolignans.

It is not known whether parent or conjugated silymarin flavonolignans are responsible for silymarin's purported therapeutic effects since both silybin and its 7-glucuronide conjugate have demonstrated antioxidant activity *in vitro* at a concentration of 330 μM (Kren et al., 2000). Recently, the anti-viral activity of a standardized silymarin extract was demonstrated in an *in vitro* cell culture model of HCV replication at concentrations ranging between 20 μM - 40 μM (Polyak et al., 2007). In our study, the peak plasma concentration for all silymarin flavonolignans combined only amounted to 0.5 μM (225 ng/ml) for HCV patients with cirrhosis, who achieved the highest levels of exposure following a customary dose of silymarin. Therefore, customary doses of silymarin are not likely to achieve the plasma concentrations required for the antioxidant and antiviral effects of silymarin.

Silymarin exposures have been underestimated in previous studies because of their failure to quantitate the six major silymarin flavonolignans. Therefore, future clinical investigations should be directed towards an evaluation of the independent roles of the major silymarin flavonolignans and their conjugates to determine their effects in various liver disease populations. However, before such studies are undertaken, pharmacokinetic studies that examine

higher, multiple daily silymarin dose regimens in patients with liver disease are needed to identify regimens that provide optimal 24 hour exposures. To this end, a Phase I double-blind, randomized clinical trial has been undertaken to evaluate the safety, tolerability, and pharmacokinetics of silymarin in a dose escalation manner in both non-cirrhotic HCV and NAFLD patients.

Acknowledgements. We thank Trang Nguyen for her assistance with sample preparation. We thank Dr. Sonia Miranda for her assistance with the caspase-3/7 and 8-isoprostane $F_{2\alpha}$ assays. We thank Dr. Heyward Hull for providing statistical consultation.

References

Bantel H, Lugering A, Poremba C, Lugering N, Held J, Domschke W, Schulze-Osthoff K (2001) Caspase activation correlates with the degree of inflammatory liver injury in chronic hepatitis C virus infection. *Hepatology* 34:758-767.

Bantel H, Lugering A, Heidemann J, Volkmann X, Poremba C, Strassburg CP, Manns MP, Schulze-Osthoff K (2004) Detection of apoptotic caspase activation in sera from patients with chronic HCV infection is associated with fibrotic liver injury. *Hepatology* 40:1078-1087.

Barnes SN, Aleksunes LM, Augustine L, Scheffer GL, Goedken MJ, Jakowski AB, Pruimboom-Brees IM, Cherrington NJ, Manautou JE (2007) Induction of hepatobiliary efflux transporters in acetaminophen-induced acute liver failure cases. *Drug Metab Dispos* 35:1963-1969.

Beckmann-Knopp S, Rietbrock S, Weyhenmeyer R, Bocker RH, Beckurts KT, Lang W, Hunz M, Fuhr U (2000) Inhibitory effects of silibinin on cytochrome P-450 enzymes in human liver microsomes. *Pharmacol Toxicol* 86:250-256.

Buzzelli G, Moscarella S, Barbagli, Marena C, Gentilini P (1994) Therapeutic effect of silipide in patients with chronic hepatic C non responders (NTs) to interferon (IFN) treatment. *J Hepatol* 21:S100.

Calabrese F, Pontisso P, Pettenazzo E, Benvegnu L, Vario A, Chemello L, Alberti A, Valente M (2000) Liver cell apoptosis in chronic hepatitis C correlates with histological but not biochemical activity or serum HCV-RNA levels. *Hepatology* 31:1153-1159.

Cermak R, Wolffran S (2006) The potential of flavonolignans to influence drug metabolism and pharmacokinetics by local gastrointestinal mechanisms. *Current Drug Metabolism* 7:729-744.

Congiu M, Mashford ML, Slavin JL, Desmond PV (2002) UDP Glucuronosyltransferase mRNA levels in human liver disease. *Drug Metab Dispos* 30:129–134.

Crocenzi FA, Roma MG (2006) Silymarin as a new hepatoprotective agent in experimental cholestasis: new possibilities for an ancient medication. *Curr Med Chem*13:1055-1074.

Ferenci P, Dragosics B, Dittrich H, Frank H, Benda L, Lochs H, Meryn S, Base W, Schneider B (1989) Randomized controlled trial of silymarin treatment in patients with cirrhosis of the liver. *J Hepatol* 9:105-113.

Gordon A, Hobbs DA, Bowden DS, Bailey MJ, Mitchell J, Francis AJ, Roberts SK (2006) Effects of silybum marianum on serum hepatitis C virus RNA, alanine aminotransferase levels and well-being in patients with chronic hepatitis C. *J Gastroenterol Hepatol* 21:275-280.

Guardigli M, Marangi M, Casanova S, Grigioni WF, Roda E, Roda A (2005) Chemiluminescence quantitative immunohistochemical determination of MRP2 in liver biopsies. *J Histochem Cytochem* 53:1451-1457.

Hinoshita E, Taguchi K, Inokuchi A, Uchiumi T, Kinukawa N, Shimada M, Tsuneyoshi M, Sugimachi K, Kuwano M (2001) Decreased expression of an ATP-binding cassette transporter, MRP2, in human livers with hepatitis C virus infection. *J Hepatol* 35:765-773.

Huber R, Futter I, Ludtke R (2005) Oral silymarin for chronic hepatitis C—a retrospective analysis comparing three dose regimens. *Eur J Med Res* 10:68–70.

Jacobs BP, Dennehy C, Ramirez G, Sapp J, Lawrence VA (2002) Milk thistle for the treatment of liver disease: a systematic review and meta-analysis. *Am J Med* 113:506-515.

Kamisako T, Ogawa H (2007) Effect of bile duct obstruction on the expression of intestinal mRNA related to cholesterol and bile acid metabolism in the rat. *J Gastroenterol Hepatol* 22:125–131.

Kren V, Ulrichova J, Kosina P, Stevenson D, Sedmera P, Prikrylova V, Halada P, Simanek V (2000) Chemoenzymatic prepration of silibin β-glucuronides and their biological evaluation. *Drug Metab Dispos* 28:1513-1517.

Kvasnicka F, Biba B, Sevcik R, Voldrich M, Kratka J (2003) Analysis of the active components of silymarin. *J Chromatogr A* 90:239-45.

Lickteig AJ, Fisher CD, Augustine LM, Aleksunes LM, Besselsen DG, Slitt AL, Manautou JE, Cherrington NJ (2007) Efflux transporter expression and acetaminophen metabolite excretion are altered in rodent models of non-alcoholic fatty liver disease. *Drug Metab Dispos* 35:1970-1980.

Lorenz D, Lucker PW, Mennicke WH, Wetzelsberger N (1984) Pharmacokinetic studies with silymarin in human serum and bile. *Methods Find Exp Clin Pharmacol* 6:655-661.

Lucena MI, Andrade RJ, de la Cruz JP, Rodriguez-Mendizabal M, Blanco E, Sanchez de la Cuesta F (2002) Effects of silymarin MZ-80 on oxidative stress in patients with alcoholic cirrhosis. Results of a randomized, double-blind, placebo-controlled clinical study. *Int J Clin Pharmacol Ther* 40:2-8.

Manna SK, Mukhopadhyay A, Van NT, Aggarwal BB (1999) Silymarin suppresses TNF-induced activation of NF-kappa B, c-Jun N-terminal kinase, and apoptosis. *J Immunol* 163:6800-6809.

Mayer KE, Myers RP, Lee SS (2005) Silymarin treatment of viral hepatitis: a systematic review. *J Viral Hepat* 12:559-567.

Morris ME, Zhang Z (2006) Flavonolignan-drug interactions: effects of flavonolignans on ABC transporters. *Life Sciences* 78:2116-2130.

Par A, Roth E, Rumi G Jr, Kovacs Z, Nemes J, Mozsik G (2000) Oxidative stress and antioxidant defense in alcoholic liver disease and chronic hepatitis C. *Orv Hetil* 141:1655-1659.

Pares A, Planas R, Torres M, Caballeria J, Viver JM, Acero D, Panes J, Rigau J, Santos J, Rodes J (1998) Effects of silymarin in alcoholic patients with cirrhosis of the liver: results of a controlled, double-blind, randomized and multicenter trial. *J Hepatol* 28:615-621.

Polyak SJ, Morishima C, Shuhart MC, Want CC, Liu Y, Lee DYW (2007) Inhibition of T cell inflammatory cytokines, hepatocyte NF-kappaB Signaling, and HCV infection by standardized silymarin. *Gastroenterology* 132:1925-1936.

Psotova J, Chlopcikova S, Grambal F, Simanek V, Ulrichova J (2002) Influence of silymarin and its flavonolignans on doxorubicin-iron induced lipid peroxidation in rat heart microsomes and mitochondria in comparison with quercetin. *Phytother Res* 16:S63-67.

Rambaldi A, Jacobs BP, Iaquinto G, Gluud C (2007) Milk thistle for alcoholic and/or hepatitis B or C virus liver diseases. *Cochrane Database Syst Rev* 4:CD003620.

Rickling, B., Hans, B., Kramarczyk, R., Krumbiegel, G., Weyhenmeyer, R (1995) Two high-performance liquid chromatographic assays for the determination of free and total silibinin

diastereomers in plasma using column switching with electrochemical detection and reversed-phase chromatography with ultraviolet detection. *J Chromatogr B Biomed Appl* 670:267-277.

Russo MW, Esposite S, Borwn Jr RS, Foulgar KC, Jacobson IM (2001) Complementary alternative medicine (CAM) use amongst patients with chronic hepatitis C infection (HCV) (abstr). *Gastroenterology* 120:A409.

Saller R, Meier R, Brignoli R (2001) The use of silymarin in the treatment of liver diseases. *Drugs* 61:2035-2063.

Schumann J, Prockl J, Kiemer AK, Vollmar AM, Bang R, Tiegs G (2003) Silibinin protects mice from T cell-dependent liver injury. *J Hepatol* 39:333-340.

Seidel N, Volkmann X, Langer F, Flemming P, Manns MP, Schulze-Osthoff K, Bantel H (2005) The extent of liver steatosis is chronic hepatitis C virus infection is mirrored by caspase activity in serum. *Hepatology* 42:113-120.

Tanamly MD, Tadros F, Labeeb S, Makld H, Shehata M, Mikhail N, Magder LS, Afdhal NH, Medhat A, Abdel-Hamid M (2004) Randomised double-blinded trial evaluating silymarin for chronic hepatitis C in an Egyptian village: study description and 12-month results. *Dig Liver Dis* 36:752–759.

Velussi, M., Cernigoi, A.M., De Monte, A., Dapas, F., Caffau, C., Zili, M (1997) Long-term (12 months) treatment with an anti-oxidant drug (silymarin) is effective on hyperinsulinemia, exogenous insulin need and malondialdehyde levels in cirrhotic diabetic patients. *J Hepatol* 26:871-879.

Wagner M, Zollner G, Fickert P, Gumhold J, Silbert D, Fuchsbichler A, Gujral JS, Zatloukal K, Denk H, Jaeschke H, Trauner M (2005) Hepatobiliary transporter expression in ICAM-/- and lpr mice after common bile duct ligation is independent of the degree of inflammation and oxidative stress. *Drug Metab Dispos* 35:1694-1699.

Wen Z, Dumas TE, Schrieber SJ, Hawke RL, Fried MW, Smith PC (2008) Pharmacokinetics and metabolic profile of free, conjugated and total silymarin flavonolignans in human plasma after oral administration of milk thistle extract. *Drug Metab Dispos* 36:65-72.

Weyhenmeyer R, Mascher H, Birkmayer J (1992) Study on dose-linearity of the pharmacokinetics of silibinin diastereomers using a new steriospecific assay. *Int J Clin Pharmacol Ther Toxicol* 30:134-138.

Wu JW, Lin LC, Hung SC, Lin CH, Chi CW, Tsai TH (2008) Effect of silibinin on the pharmacokinetics of pyrazinamide and pyrazinoic acid in rats. *Drug Metab Dispos* 36:589-596.

Footnote

This work was supported by the National Institute of Health grants # K24 DK066144 and # RR00046, from the General Clinical Research Centers program of the Division of Research Resources.

FIGURE LEGENDS

Figure 1. Total (parent + conjugated) concentration vs time profiles of the six major silymarin flavonolignans in healthy volunteers (Panel A) and HCV patients with cirrhosis (Panel B).

Panel A contains the legend for Figure 1. The linear quantitative range for the six silymarin flavonolignans by the LC-MS assay is 5 - 1000 ng/ml.

Figure 2. Sum silymarin conjugates vs time profile for each cohort.

Table inset depicts the geometric means (95% CI) for Sum silymarin conjugates $AUC_{0\text{-}24h}$, (expressed as "Parent Flavonolignan Equivalents"), $^{*}p \leq 0.03$, comparisons to the healthy cohort.

Figure 3. The percent (%) of total conjugates that are glucuronides for silybin B and isosilybin B at 1.5- hrs post-dose.

Bars represent the cohorts: healthy, open; HCV noncirrhotic, gray; cirrhotic cohort, black; and NAFLD, white-hatched.

Figure 4. Correlation of Sum silymarin conjugates $AUC_{0\text{-}24h}$ (expressed as "Parent Flavonolignan Equivalents") with measures of liver disease activity among all study participants.

Caspase 3/7 activity correlated with Sum silymarin conjugates $AUC_{0\text{-}24h}$ (R^2=0.52, $p<0.001$). Plasma 8-isoprostane $F_{2\alpha}$ did not correlate with Sum silymarin conjugates $AUC_{0\text{-}24h}$ (R^2=0.01, $p>0.9$), see Figure inset. RLU, relative light units.

DMD Fast Forward. Published on June 19, 2008 as DOI: 10.1124/dmd.107.019604
This article has not been copyedited and formatted. The final version may differ from this version.

Table 1. Subject characteristics.

	Healthy	Non Cirrhotic	Cirrhotic	NAFLD
Male : Female, n	3 : 2	3 : 2	3 : 2	1 : 4
White : Black, n	5 : 0	5 : 0	4 : 1	4 : 1
Age, years	30 ± 16.9	51.2 ± 8.2	52.6 ± 3.6	57 ± 9.2
Weight, kg	76.5 ± 6.3	80.7 ± 11.0	88.4 ± 12.8	76.2 ± 12.8
BMI, kg/m^2	24.2 ± 2.6	28.9 ± 2.9	29.2 ± 3.6	28.6 ± 4.9
Biopsy stage range	n/a	0-2	3-4	0-3
ALT, U/L	35 ± 3	75 ± 33	290 ± 392	85 ± 42
Platelet count, $cells/mm^3$	266 ± 55	306 ± 90	123 ± 64	248 ± 50
Total bilirubin, mg/dl	0.34 ± 0.21	0.43 ± 0.30	0.86 ± 0.38	0.52 ± 0.37

Data are presented as mean values ± standard deviation unless otherwise specified.

BMI, body mass index.

DMD Fast Forward. Published on June 19, 2008 as DOI: 10.1124/dmd.107.019604
This article has not been copyedited and formatted. The final version may differ from this version.

Table 2. Pharmacokinetics of parent silymarin flavonolignans.

		SA	SB	ISA	ISB	SC	SD
Healthy	C_{max}	12	9	3	n.d.	n.d.	n.d.
	(ng/ml)	(2, 67)	(2, 43)	(1, 14)			
	$AUC_{0\text{-}24h}$	33	23	3	n.d.	n.d.	n.d.
	(ng*h/ml)	(2, 488)	(2, 302)	(1, 16)			
	CL/F	970	2354	2835[a]	n.d.	n.d.	n.d.
	(L/h)	(168, 5590)[c]	(308, 18034)[c]				
Non-	C_{max}	13	12	5	5	9	4
Cirrhotic	(ng/ml)	(1, 269)	(1, 252)	(0.3, 86)	(0.3, 85)	(1, 119)	(0.4, 39)
	$AUC_{0\text{-}24h}$	13	16	5	5	13	5
	(ng*h/ml)	(1,156)	(1, 397)	(0.4, 57)	(0.3, 63)	(1, 244)	(0.3, 90)
	CL/F	713	1581	720[a]	281[a]	1095	484[a]
	(L/h)	(344, 1488)[b]	(584, 4316)[b]			(508, 2357)[b]	

DMD Fast Forward. Published on June 19, 2008 as DOI: 10.1124/dmd.107.019604
This article has not been copyedited and formatted. The final version may differ from this version.

Table 2 (continued). Pharmacokinetics of parent silymarin flavonolignans.

		SA	SB	ISA	ISB	SC	SD
Cirrhotic	C_{max}	69	33	11	15	5	n.d.
	(ng/ml)	(45, 107)	(3, 385)	(1, 156)	(2, 104)	(0.3, 91)	
	AUC_{0-24h}	41	149	12	16	7	n.d.
	(ng*h/ml)	(3, 549)	(115, 195)	(1, 191)	(2, 115)	(0.2, 251)	
	CL/F	509	1156	505	402	541 [a]	n.d.
	(L/h)	(389, 667)	(614, 2165)[c]	(208, 1222)[b]	(240, 675)[c]		
NAFLD	C_{max}	61	40	n.d.	5	n.d.	n.d.
	(ng/ml)	(24, 157)	(14, 110)		(0.3, 62)		
	AUC_{0-24h}	40	84	n.d.	4	n.d.	n.d.
	(ng*h/ml)	(19, 43)	(43, 166)		(0.4, 51)		
	CL/F	904	3019	n.d.	349[a]	n.d.	n.d.
	(L/h)	(454, 1800)	(1422, 6438)				

Data are presented as geometric means (95% CI). Not determined (n.d.) indicates concentrations below limit of quantitation. Geometric means for CL/F reflect n=5 unless otherwise specified: [a]the average of only two values, [b]n=3, [c]n=4.

DMD Fast Forward. Published on June 19, 2008 as DOI: 10.1124/dmd.107.019604
This article has not been copyedited and formatted. The final version may differ from this version.

Table 3. Pharmacokinetics of total (parent + conjugated) silymarin flavonolignans.

		SA	SB	ISA	ISB	SC	SD
Healthy	C_{max}	37	106	71	41	37	12
	(ng/ml)	(23, 61)	(63, 177)	(32, 157)	(21, 80)	(18, 76)	(2, 64)
	AUC_{0-24h}	256	617	491	251	355	51
	(ng*h/ml)	(118, 557)	(327, 1164)	(243, 993)	(145, 436)	(169, 745)	(3, 807)
Non	C_{max}	73	269	131	76	144	39
Cirrhotic	(ng/ml)	(19, 282)	(73, 995)	(40, 422)	(21, 274)	(49, 423)	(12, 131)
	AUC_{0-24h}	301	1195	858	494	1699	228
	(ng*h/ml)	(100, 901)	(374, 3823)	(282, 2610)	(154, 1588)	(633, 4564)*	(82, 634)
Cirrhotic	C_{max}	151	551	339	193	147	75
	(ng/ml)	(71, 319)*	(309, 982)*	(160, 720)*	(110, 338)*	(68, 318)*	(33, 171)*
	AUC_{0-24h}	685	2899	2231	1254	1841	507
	(ng*h/ml)	(265, 1776)*	(1082, 7767)*	(641, 7760)*	(478, 3287)*	(802, 4226)*	(167, 1542)
NAFLD	C_{max}	124	430	216	137	190	68
	(ng/ml)	(56, 278)*	(215, 860)*	(96, 485)	(55, 343)*	(100, 361)*	(43, 108)*
	AUC_{0-24h}	521	1720	1279	706	2043	352
	(ng*h/ml)	(342, 795)	(943, 3137)	(690, 2371)	(406, 1230)	(1170, 3566)*	(252, 493)

Data are presented as geometric means (95% CI), * $p \leq 0.02$, comparisons to the healthy cohort.

Figure 1-Panel A.

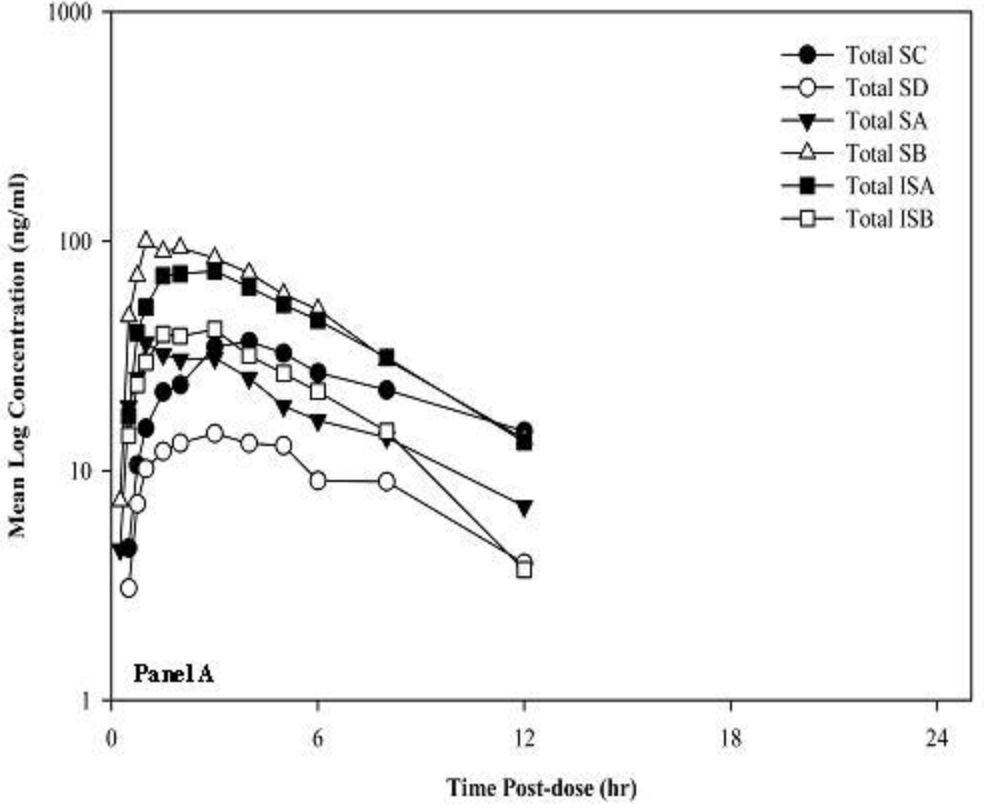
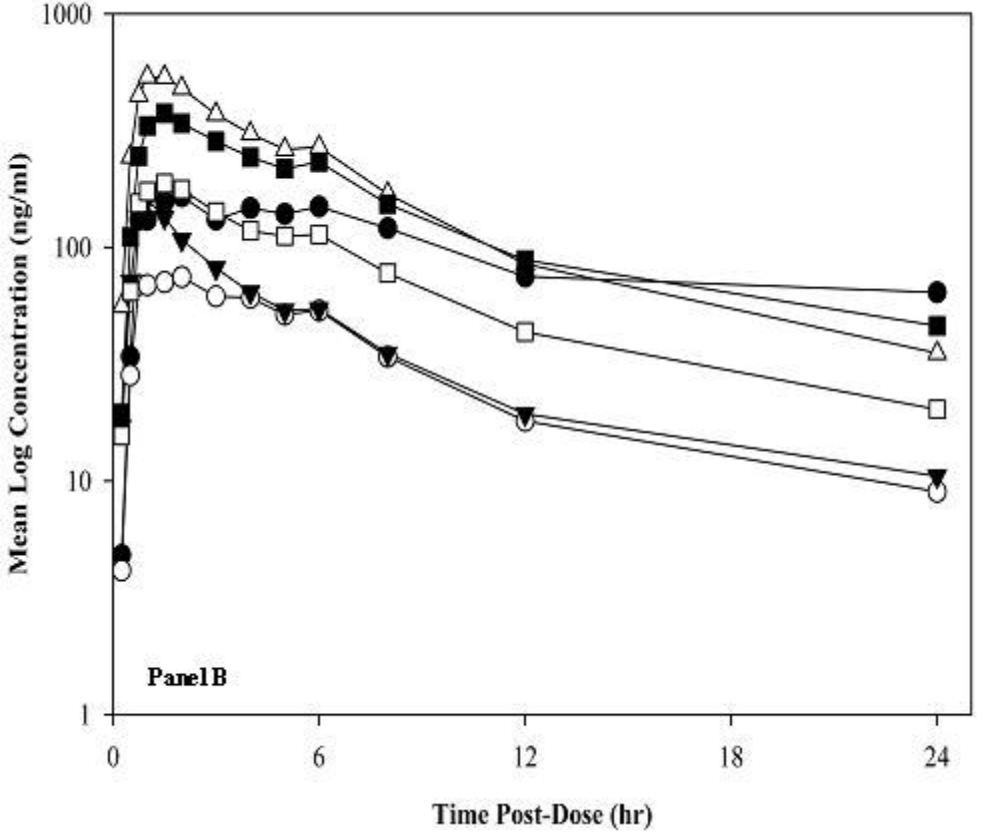


Figure 1-Panel B.



DMD Fast Forward. Published on June 19, 2008 as DOI: 10.1124/dmd.107.019604
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 2.

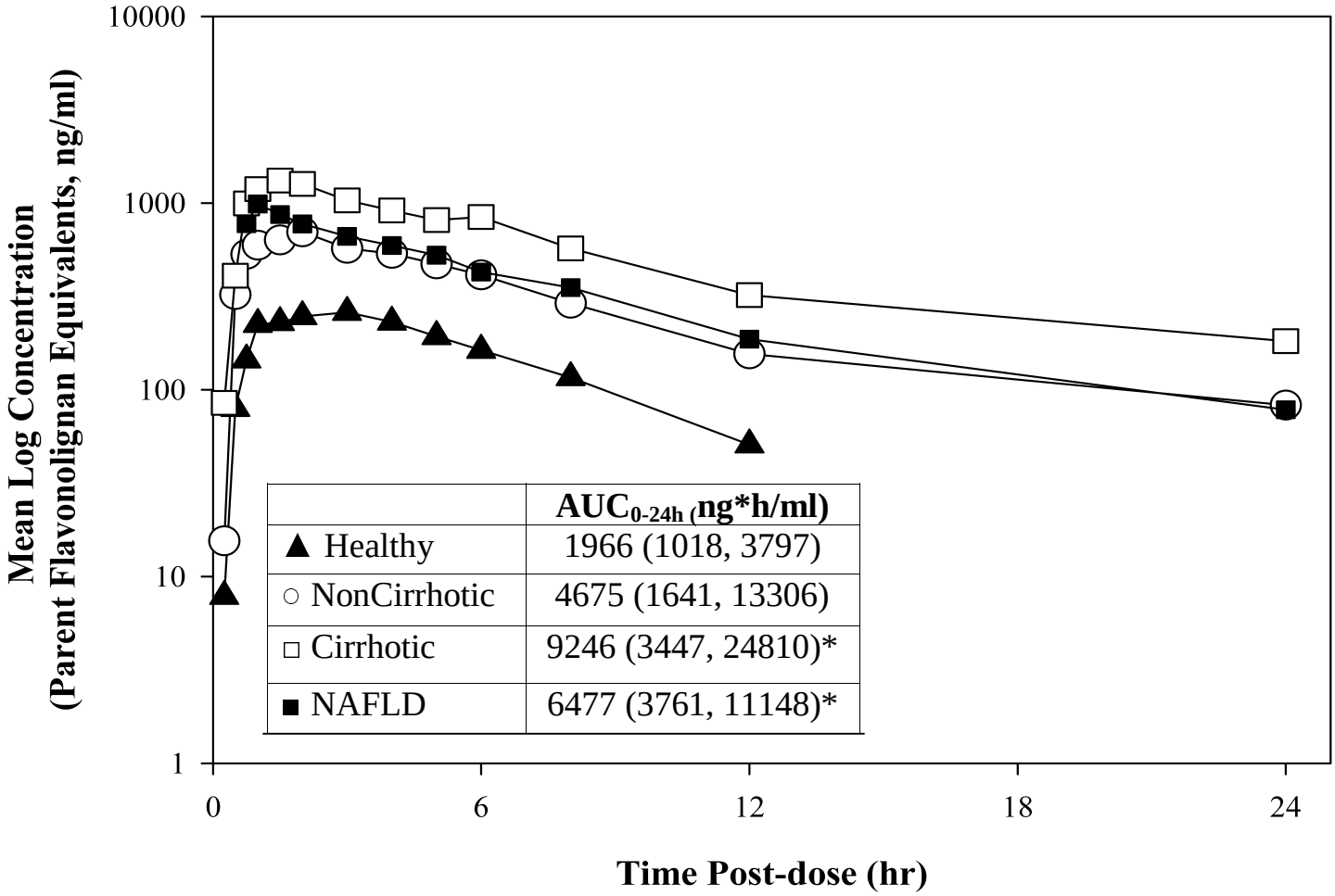


Figure 3.

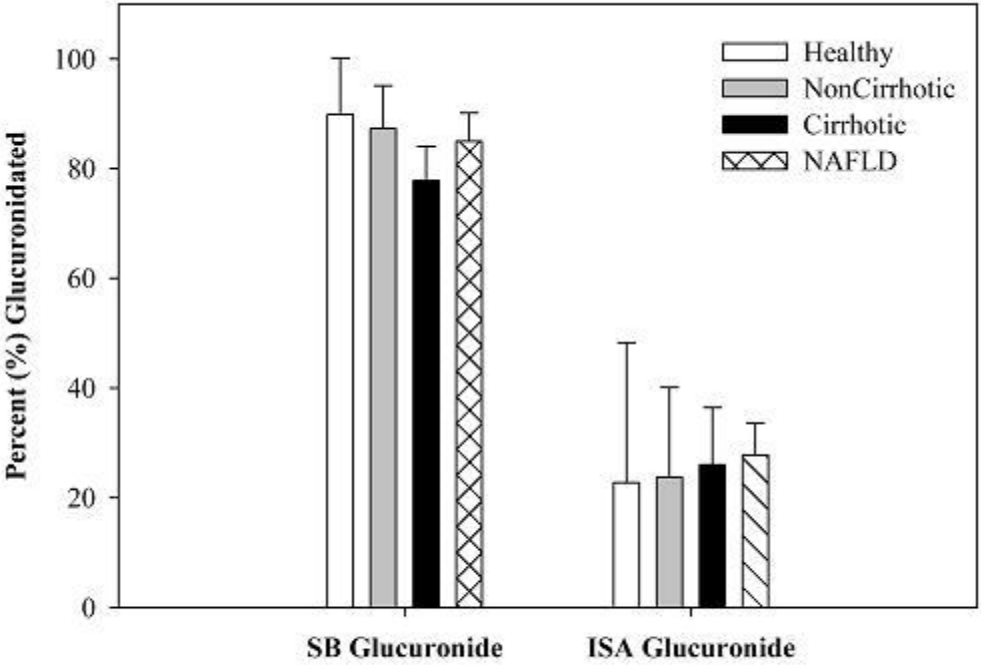
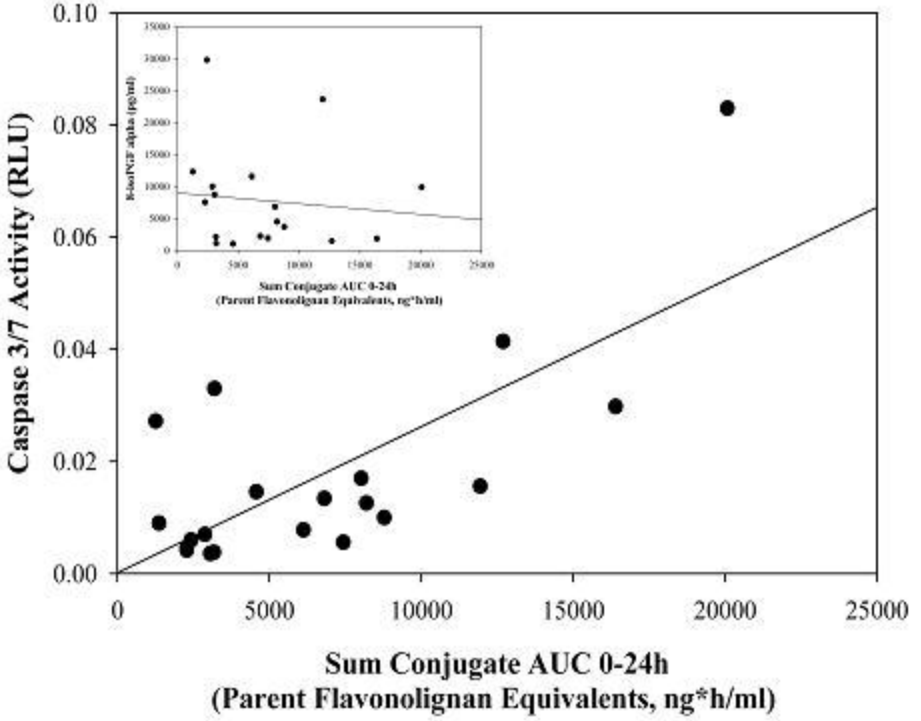

Healthy
NonCirrhotic
Cirrhotic
NAFLD
Percent (%) Glucuronidated
100
80
60
40
20
0
SB Glucuronide
ISA Glucuronide

Figure 4.


Caspase 3/7 Activity (RLU)
0.10
0.08
0.06
0.04
0.02
0.00
0
5000
10000
15000
20000
25000
Sum Conjugate AUC 0-24h
(Parent Flavonolignan Equivalents, ng*h/ml)
Sum Conjugate AUC 0-24h
(Parent Flavonolignan Equivalents, ng*h/ml)