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File: ■ Milk Thistle (*Silybum marianum*)
■ Silymarin
■ Liver Disease

HC 111162-443

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RE: Milk Thistle Flavonolignan Plasma Levels Greater in Patients with Non-alcoholic Fatty Liver Disease than in Those with Hepatitis C

Schrieber SJ, Hawke RL, Wen Z, et al. Differences in the disposition of silymarin between patients with nonalcoholic fatty liver disease and chronic hepatitis C. *Drug Metab Dispos*. 2011 Dec;39(12):2182-2190.

Silymarin, a concentrated mixture of 6 flavonolignans isolated from milk thistle (*Silybum marianum*) seed, includes silybins A and B, isosilybins A and B, silychristin, and silydianin. Silymarin has been used traditionally for treating liver diseases, and its antioxidant activity is thought to help with liver conditions involving oxidative stress. Additionally, the severity of liver disease has been shown to impact the pharmacokinetics (absorption, metabolism, excretion, etc.) of silymarin. As oxidative stress is associated with the progression of non-alcoholic fatty liver disease (NAFLD) and hepatitis C virus (HCV), it is hypothesized that silymarin will have a beneficial effect on patients suffering from these diseases. To address potential differences in the effect of silymarin in patients with varying forms of liver diseases, this study analyzed silymarin flavonolignan pharmacokinetics in NAFLD and HCV patients enrolled in a single-blind Phase I clinical trial.

Included patients (n=40) were males and females over 18 years old that suffered from chronic noncirrhotic NAFLD and HCV as characterized by both alanine aminotransferase concentrations at or over 65 IU/L within a year prior to the study and a screening creatinine clearance greater than 60 ml/min. Patients were excluded if they had other chronic or decompensated liver disease, cirrhosis, or steatohepatitis (type of liver disease characterized by inflammation of the liver with concurrent fat accumulation in the liver); human immunodeficiency virus (HIV); allergy to or consumption of milk thistle; or the use of silymarin or antioxidants in high doses or the use of more than 2 g per day of acetaminophen, oral contraceptives, warfarin, metronidazole, or cytochrome P450 3A4 (CYP3A4) inducers. Also, those who abused drugs, consumed more than 12 g per day of alcohol, or were pregnant or breast-feeding were excluded. All patients were asked to refrain from alcohol 48 hours before the study start through the study's conclusion.

The study was carried out from December 2006 to July 2008 at the University of North Carolina at Chapel Hill, North Carolina; Beth Israel Deaconess Medical Center, Boston, Massachusetts; and the University of Pennsylvania and Thomas Jefferson University, both in Philadelphia, Pennsylvania. Patients were randomized to disease/dosage groups (n=8) to receive either treatment or placebo (in a 3:1 ratio) every 8 hours for 7 days. The 48-hour measurements were done with a single-dose treatment prior to the 7-day treatment, and after the study's completion when a final dose was administered. Treatments were taken 30 min after breakfast with 240 ml of water after an overnight fast; the meal was chosen by patients from a predetermined menu that excluded grapefruit juice (which is known to interact with a wide range of medications, increasing their serum levels). Blood samples were collected 0, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 15, 18, 24, 32, and 48 hours after treatment was administered.

Patients stayed at the clinic for 48 hours after the single dose and were sent home with treatment for the 7-day study. Patients were asked to keep a diary of treatment compliance, and pills were counted at the end of the study. Blood tests and a questionnaire addressing symptoms and severity of any adverse side effects were used to assess safety; these were both administered on days 1, 6, 8, and 10 of the study.

The treatment consisted of capsules of the silymarin preparation Legalon® (Madaus Rottapharm Group; Cologne, Germany), the world's leading clinically tested milk thistle preparation. Legalon is standardized to contain 70.8% of total flavonolignans comprised of 23.2 mg of silybin A, 32.0 mg of silybin B, 11.8 mg of isosilybin A, 6.6 mg of isosilybin B, 24.9 mg of silychristin, and 29.0 mg of silydianin. Patients using silymarin took either 280 mg or 560 mg of Legalon with or without placebo capsules, respectively.

In assessing adverse side effects, the blood tests showed no abnormalities associated with Legalon consumption. Of the effects reported in the HCV patients, only dizziness was considered connected with Legalon and cleared up in a day. Adverse side effects in the NAFLD patients were considered "mild to moderate" and not associated with Legalon.

When comparing the pharmacokinetics of a single dose of either 280 or 560 mg of Legalon, silybin A was found to have a larger maximum plasma concentration (C_{max}) and area under the curve over 48 hours (AUC_{0-48h}) than silybin B. There were no significant differences observed in the pharmacokinetics of silybins A and B between the HCV patients as compared to the NAFLD patients at the 280 mg dose; however, at the 560 mg dose, the AUC_{0-48h} of silybin A was 1.5-fold greater ($P>0.05$) and silybin B was significantly 2.1-fold greater ($P<0.05$) in NAFLD patients than HCV patients, respectively.

In the comparison of the pharmacokinetics of silybins A and B in the first 8 hours after the final dose following the 7-day treatment period, silybin A was also found to have a 2.1- to 3.6-fold greater C_{max} and a 2.6- to 4.9-fold greater AUC_{0-8h} than silybin B. Patients in both HCV and NAFLD groups showed significantly greater AUC_{0-8h} with the 560 mg dose than the 280 mg dose for both compounds when adjustments for weight and disease type were done ($P\leq0.004$). Additionally, the adjusted mean for the AUC_{0-8h} of silybin B was greater in NAFLD patients than those in the HCV group when adjusted for both weight and dose ($P=0.004$).

The conjugates of silybins A and B were also compared across dosage levels and diseases; both C_{max} and AUC_{0-8h} were found to be greater for silybin B conjugates as

compared to silybin A conjugates. At the 280 mg dose, the C_{max} and $AUC_{0\text{-}8h}$ of silybin B in NAFLD patients were both significantly lower as compared to the HCV patients ($P<0.05$ for both). In addition, it was found that silybin B conjugates were lower in NAFLD patients as compared to HCV patients over the entire 8 hours; however, the levels of unmetabolized silybin B were higher in the NAFLD group only until a decline after peak concentrations when the levels became similar between both disease groups.

The $AUC_{0\text{-}8h}$ was significantly greater at the 560 mg dosage as compared to the 280 mg dosage level for both silybin A and B conjugates when adjusted for weight and disease type ($P\leq0.004$). Additionally, the metabolic ratio of the $AUC_{0\text{-}8h}$ for silybin B to silybin B conjugates at the 560 mg dose was 4-fold greater in NAFLD patients than HCV patients (0.060 ± 0.041 vs. 0.016 ± 0.011, respectively; $P<0.05$).

No significant accumulation was reported for either silybin A or B. The steady-state peak concentrations of isosilybin A and B, silychristin, and silydianin at the 560 mg dose were significantly higher in NAFLD patients as compared to HCV patients [P values not reported]; silychristin and silydianin could not be detected at all in HCV patients. In addition to the absorption peak at the 1-hour timepoint after the 560 mg dose, the 6 flavonolignans showed a secondary peak at the 4-hour timepoint (and most had a third peak after 8 hours) in NAFLD patients but not in HCV patients, indicating significant enterohepatic cycling in the NAFLD group.

In summary, it is asserted that drug metabolism in different liver diseases is not well researched. Previous investigations have shown that silymarin flavonolignans have differences in metabolism and transport that may explain the varying AUC values among patients with NAFLD and HCV. The evidence of enterohepatic cycling in NAFLD patients but not in HCV patients may be due to functional differences of hepatobiliary transporters associated with these liver diseases. The differences in the pharmacokinetics of silymarin between the diseases may also be caused by disease-specific liver function alterations. In support of this conclusion, the authors' previous work has shown significant differences of silymarin conjugates between patients with NAFLD and HCV as compared to healthy subjects. Information on the pharmacokinetics of this widely used liver support supplement is important not only to understand the relative flavonolignan bioavailability and proper dosing for those with liver diseases, but also to inform the ongoing Phase II clinical trial for which this study provided critical oral dosing data.

—Amy C. Keller, PhD

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