

A Randomized, Controlled, Double-Blind, Pilot Study of Milk Thistle for the Treatment of Hepatotoxicity in Childhood Acute Lymphoblastic Leukemia (ALL)

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Abstract:

BACKGROUND: Despite limited preclinical and clinical investigations, milk thistle (MT) is often used for the treatment of chemotherapy-associated hepatotoxicity. Limited treatment options exist for chemotherapy-related hepatotoxicity. Given the wide use of MT, the authors investigated MT in both the laboratory and a clinical setting.

METHODS: In a double-blind study, children with acute lymphoblastic leukemia (ALL) and hepatic toxicity were randomized to MT or placebo orally for 28 days. Liver function tests were evaluated during the study period. To assess MT in vitro, the authors evaluated supratherapeutic concentrations in an ALL cell line.

RESULTS: Fifty children were enrolled. No significant differences in frequency of side effects, incidence and severity of toxicities, or infections were observed between groups. There were no significant changes in mean amino alanine transferase (ALT), aspartate amino transferase (AST), or total bilirubin (TB) at Day 28. At Day 56, the MT group had a significantly lower AST ($P = .05$) and a trend toward a significantly lower ALT ($P = .07$). Although not significantly different, chemotherapy doses were reduced in 61% of the MT group compared with 72% of the placebo group. In vitro experiments revealed no antagonistic interactions between MT and vincristine or L-asparaginase in CCRF-CEM cells. A modest synergistic effect with vincristine was observed.

CONCLUSIONS: In children with ALL and liver toxicity, MT was associated with a trend toward significant reductions in liver toxicity. MT did not antagonize the effects of chemotherapy agents used for the treatment of ALL. Future study is needed to determine the

most effective dose and duration of MT and its effect on hepatotoxicity and leukemia-free survival.

Keywords: acute lymphoblastic leukemia | hepatotoxicity | milk thistle | complementary medicine | childhood cancer

Article:

During the past 2 decades, there has been an increased interest in understanding the mechanisms and clinical applications of milk thistle (*Silybum marianum*), an herbal plant.¹⁻³ Prior studies have found milk thistle (MT) has both hepatoprotectant and nephroprotectant activity, thus suggesting its application as a supportive care agent.⁴ MT is available in the United States as a dietary supplement and most often is used for its effects on the liver. Clinical studies have investigated MT for the prevention or treatment of liver damage in patients with hepatitis and cirrhosis.^{3,5} A case report suggested that MT plays a beneficial role for the treatment of chemotherapy-induced hepatotoxicity.⁶

In the treatment of children with acute lymphoblastic leukemia (ALL), the administration of chemotherapy agents is frequently interrupted because of liver toxicity, especially during the maintenance phase of treatment. Schmiegelow et al found that children with ALL who experience a cumulative withdrawal of methotrexate or 6 mercaptopurine of greater than 10% of the prescribed therapy have an increased risk of bone-marrow relapse (methotrexate: complete hematologic remission [CHR], $45\% \pm 12\%$ versus $78\% \pm 5\%$, $P = .009$; 6 mercaptopurine: CHR $31\% \pm 12\%$ versus $77\% \pm 5\%$, $P < .00,001$).⁷ Hepatotoxicity was the main reason for cumulative, long-term, drug withdrawals. More recent investigations have found that 66.5% of children with ALL encountered grade 2 or higher liver toxicity at some point during their therapy.⁸

Currently, there are no substitute chemotherapy agents that provide the same effectiveness against ALL yet preserve liver function. There are also no hepatoprotective medications that allow chemotherapy to continue to be administered while preserving liver function. Thus, adjunctive agents that may enable optimal doses of chemotherapy to be administered without necessitating a decrease in the recommended doses of chemotherapy are of clinical significance and may further improve survival in children with ALL. We present the results from a multicenter pilot study that evaluated the safety and feasibility of MT for the treatment of hepatotoxicity in children with ALL who were receiving maintenance-phase chemotherapy.

MATERIALS AND METHODS

In Vitro Antileukemic Cytotoxicity Assay

MT (Siliphos; Thorne Research, Sandpoint, ID) is a 1:2 mixture of silibinin, the most active fraction of milk thistle, and soy phosphatidylcholine, a formulation reported to improve the bioavailability of milk thistle.⁹ Silibinin is a mixture of 2 diastereoisomeric compounds, Silybin A and B. Silibinin was investigated for potential antagonism of the in vitro cytotoxic effects of selected agents used in ALL treatment regimens. CCRF-CEM T-cell ALL cells were

concurrently exposed to a dose range of either vincristine or L-asparaginase in the presence of a constant concentration of 0, 10, or 30 μM silibinin for 72 hours. These silibinin concentrations were chosen to represent at least a 10-fold higher concentration than the anticipated C_{max} in the clinical trial. The described in vitro experiments followed previously described methodology.¹⁰ Mean percentage survival (+standard error of the mean[SD]) for each treatment group was calculated relative to the mean absorbance of vehicle-treated controls.

Clinical Trial

Eligibility

This study was approved by the institutional review boards of all participating institutions. To meet eligibility requirements, children with ALL were between the ages of 1 and 21 years, were in the maintenance phase of therapy, and were treated according to the Children's Cancer Group or the Children's Oncology Group,^{11, 12} or the Dana Farber Cancer Institute ALL Consortium protocols (Fig. 1).

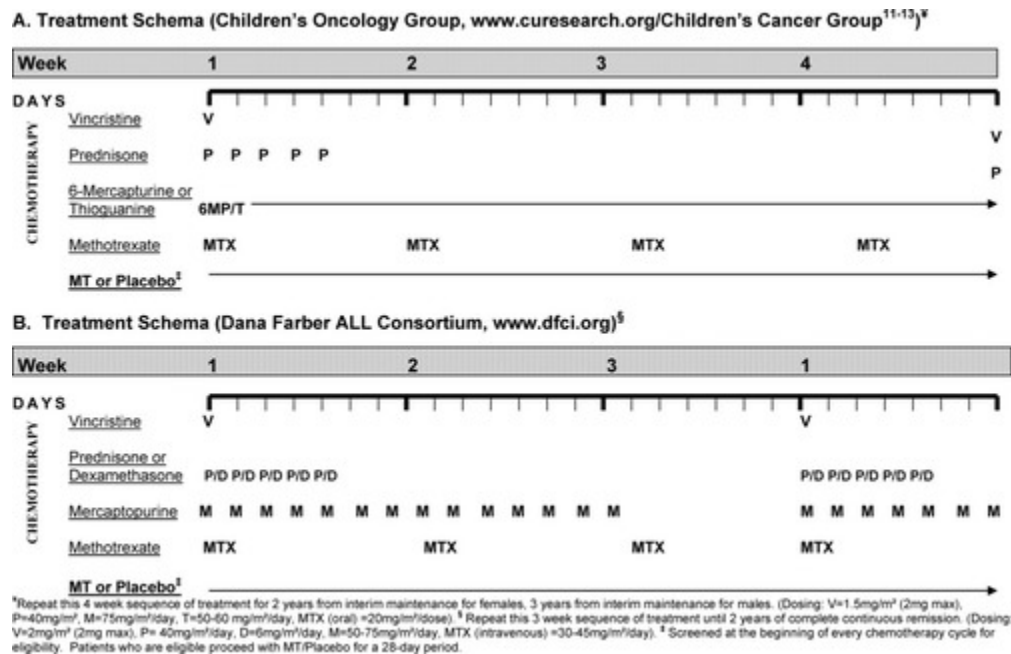


Figure 1. *Repeat this 4 week sequence of treatment for 2 years from interim maintenance for females, 3 years from interim maintenance for males. (Dosing: V = 1.5 mg/m²(2 mg max), P = 40 mg/m², M = 75 mg/m²/day, T = 50-60 mg/m²/day, MTX (oral) = 20mg/m²/dose). †Repeat this 3 week sequence of treatment until 2 years of complete continuous remission. (Dosing: V = 2 mg/m² (2 mg max), P = 40mg/m²/day, D = 6 mg/m²/day, M = 50-75 mg/m²/day, MTX (intravenous) = 30-45 mg/m²/day). ‡Screened at the beginning of every chemotherapy cycle for eligibility. Patients who are eligible proceed with MT/Placebo for a 28-day period.

During the maintenance phase of therapy, patients are routinely evaluated for liver toxicity at the beginning of each cycle of chemotherapy. Children were eligible for participation when they had hepatic toxicity of grade 2 or greater (National Cancer Institute, Common Toxicity Criteria, Version 2.0)¹³ on any 1 of the following 3 tests, amino alanine transferase (ALT), aspartate

amino transferase (AST), or total bilirubin (TB). Patients with extrahepatic biliary obstruction, severe hepatic and/or kidney failure, gastrointestinal obstruction, or malabsorption syndromes were excluded from participation.

Study design

The study was a randomized, double-blind, placebo-controlled trial (Fig. 2). Upon informed consent and assent, patients were randomly selected to receive the study drug, milk thistle, or a placebo by mouth daily for 28 days. Supplementation with milk thistle began the day after administration of intravenous chemotherapy. Hepatic toxicity was measured at Day 0, Day 28, and Day 56. To monitor adherence, patients were contacted weekly by telephone interviews and were asked to return medication containers. Adherence was defined as having completed at least 80% of the assigned drug or placebo.

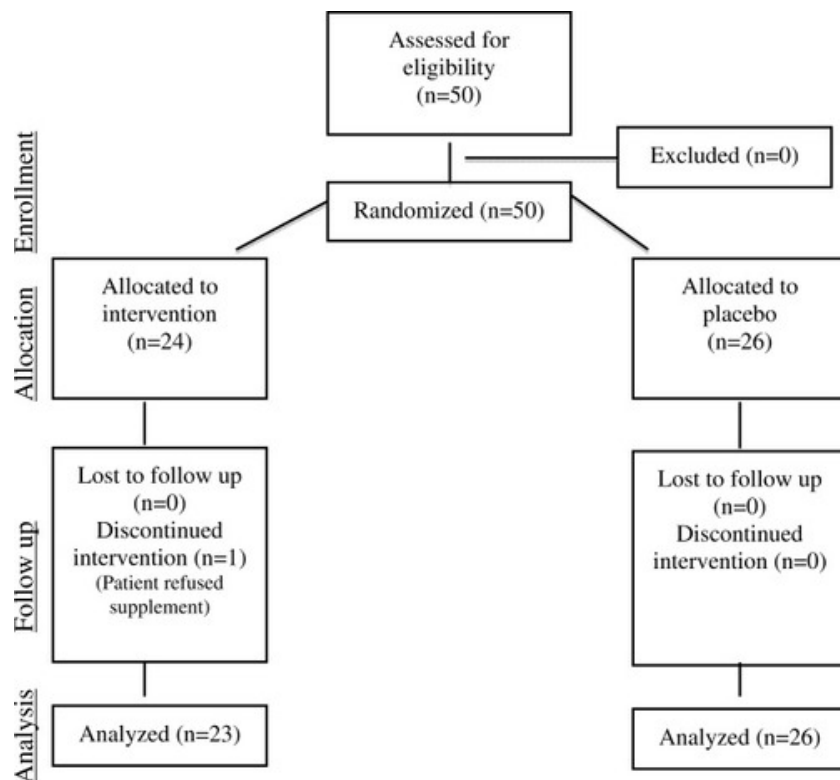


Figure 2. The study design is depicted.

Safety monitoring

The safety of MT was monitored by patient visits, chart reviews, and patient reports. We monitored for published side-effects associated with administration of MT.¹⁴ A safety monitoring rule was incorporated into the statistical analysis to screen for rates of grade 3 and 4 toxicities. We also monitored for any unexpected side effects related to MT through weekly telephone contact by our research assistant who used a standardized questionnaire that was administered to the patient or primary guardian during the intervention period.

Milk thistle

Each MT capsule contained 240 mg of milk thistle, standardized to 80 mg of silibinin (Silybin A and B). The target dose of silibinin was 5.1 mg/kg/day. Given capsule sizes and a wide range in the body weight of study participants, the following dose ranges were prescribed: patients weighing 15-20 kg received 80 mg/day; 21-40 kg patients received 160 mg/day; 41-60 kg patients received 240 mg/day; and 61-70 kg patients received 320 mg/day.

Each patient was instructed to take the capsule or its contents by mouth. Patients who opened capsules were instructed to take the contents either mixed with liquid or food and to use a spoon for administration. The placebo was identical in appearance and odor to the active agent. The study agent and placebo were stored and administered by the research pharmacy. Only the research pharmacist had knowledge of each patient's assignment to drug or placebo by random selection.

Purity and stability analysis

Both the MT (Siliphos) and the placebo were donated by Thorne Research (Sandpoint, ID). A certificate of analysis accompanied each shipment of MT and placebo. To ensure purity, content, and stability, MT and placebo capsules were analyzed independently at the Natural Products Laboratory at the Research Triangle Institute (Research Triangle Park, NC) by using previously described methodology.^{10, 15} MT was assessed at study initiation and at the study's midpoint. A slight but consistent overfill of each MT capsule was observed (281.6 ± 3.7 mg) with each MT capsule containing a total of 97.0 mg (± 5.5 mg); silibinin presented as 42.4 mg (± 2.2 mg) Silybin A and as 54.6 mg (± 3.2 mg) Silybin B. Stability of $>98\%$ was observed at 21 months.

Plasma silibinin

Plasma silibinin levels were evaluated in the subset of patients who had reported taking the final dose of MT or placebo within 2 hours before their routine blood draw. Plasma silibinin levels were evaluated at each time point by using previously described methodology.¹⁶ The limit of the detection for each spiked sample was 15 ng/mL (0.031 μ M) with an average recovery of 52%. The maximal sensitivity of the assay was 0.06 μ M for each compound or approximately 0.12 μ M for the silibinin mixture.

Statistical Methods

Demographic information and eligibility criteria were summarized by using descriptive statistics for both the MT and the placebo groups. The 2 groups were compared on these variables by using either the 2-sample Student *t* test or the chi-square test (or Fisher exact test for sparse data).

The main analysis compared groups on liver toxicity, measured by aspartate amino transferase (AST) and amino alanine transferase (ALT). A 2-sample Student *t* test was used to compare mean AST and ALT levels by group at each time point and to compare the groups on mean change in AST or ALT from baseline to Day 28 and from baseline to Day 56. We analyzed the changes in total bilirubin (TB) by comparing the percentages of patients with greater than 50%

reduction in TB in the 2 groups by using the chi-square test. The analyses were conducted with SAS version 9.1 software (Cary, NC). A *P*-value of .05 or less was considered to be statistically significant.

Sample size justification

The primary analysis endpoint was evidence of hepatic toxicity, measured by the liver function tests, ALT, AST, TB at Day 28 and Day 56. Data from previous studies suggest that liver enzyme measurements are more nearly normally distributed on the logarithmic scale. To detect a mean difference from pretreatment to post-treatment liver enzymes of 50%, or 0.7 U on the log scale, assuming log-scale SD to be 0.7, a 2-sample Student *t* test has 90% power when there are at least 23 patients per study arm. To account for possible data losses, we enrolled 25 patients per arm.

RESULTS

In Vitro

Silibinin did not antagonize the activity of vincristine or L-asparaginase in vitro when using the CCRF CEM cell line because no significant reduction in cytotoxicity was observed. We observed a degree of antileukemic synergy between silibinin and vincristine. Fixed concentration-ratio experiments and cell-survival data revealed a modest degree of synergism between vincristine and silibinin as observed by Chou-Talalay¹⁷; combination indices ranging from 0.38 to 0.62 ($P < .05$) over a 20-fold concentration range of the 2 agents (<1.0, synergy; 1.0, simple additivity, >1.0, antagonism). No such effect was observed with L-asparaginase and silibinin. Silibinin did not antagonize vincristine or L-asparaginase-mediated cell death in T-cell ALL cell culture studies.

Clinical Study

Patients

Informed consent was obtained for the 50 children enrolled from May 2002 to August 2005, with 26 patients randomly assigned to the placebo arm and 24 to the MT arm. Of the 50 children, 49 were evaluable. One parent withdrew her child from participation because the child refused to take any dose of MT. This patient was excluded from the study analysis.

Baseline characteristics by group assignment are described in Table 1. The mean ages were 8.7 and 7.0 years for the MT and placebo groups, respectively. Of the 50 children, 58% were males, and more patients were in the standard-risk group (64%) compared with the high-risk group. No significant differences for any demographic variable were observed.

Eligibility and dosing

Most patients (76%) were enrolled because of elevated ALT (19 per group). In the MT group, 83% of patients and, in the placebo group, 96% of patients experienced a grade 2 toxicity; 17%

experienced a grade 3 toxicity in the MT group and 4% in the placebo group. No patients were identified for participation due to a grade 4 toxicity. Four patients in the MT group and 10 patients in the placebo group received 1 capsule (80 mg) per day; 10 patients in the MT group and 13 patients in the placebo group received 2 capsules (160 mg) per day; 5 in MT and 3 in placebo received 3 capsules (240 mg) per day; and 4 in the MT and 2 in the placebo received 4 capsules (320 mg) per day.

Table 1. Demographics by Randomized Group

Variable	Milk Thistle (n=24)	Placebo (n=26)
Age, y		
Mean	8.7 ± 5.1	7 ± 3.2
Median	7.8	6.2
Range	1.7-18.9	2-14.3
Gender		
Male	14	15
Female	10	11
Race		
Caucasian	15	19
Black, Not Hispanic	1	0
Hispanic	6	4
Other	2	3
Risk Group		
Standard risk	15	17
High risk	9	9
Eligibility (elevated)		
AST	0	0
ALT	19	19
AST and ALT	3	4
TB	1	1
AST, ALT, TB	1	2

ALT indicates amino alanine transferase; AST, aspartate amino transferase; TB, total bilirubin.

Silibinin plasma levels

We analyzed plasma silibinin levels in 18 patients at each time point (9 patients in each group). Plasma samples were processed for quantification of the silibinin components, Silybin A and Silybin B. Although our limit of detection was at least 10-fold below the concentration reported in publications,¹⁶ detectable levels of silibinin compounds were not observed in any of the plasma samples from our study patients.

Evaluation of Liver Toxicity

We investigated changes in mean transaminase and bilirubin levels in the MT and placebo groups over the course of the study period. The mean levels of AST, ALT, and TB at Day 0, Day 28, and Day 56 for the treatment and placebo groups are presented in Figure 3A-C. Mean

baseline values did not differ significantly between the groups for AST, ALT, or TB at baseline or at Day 28. At Day 56, the MT group had lower AST than the placebo group ($P = .04$).

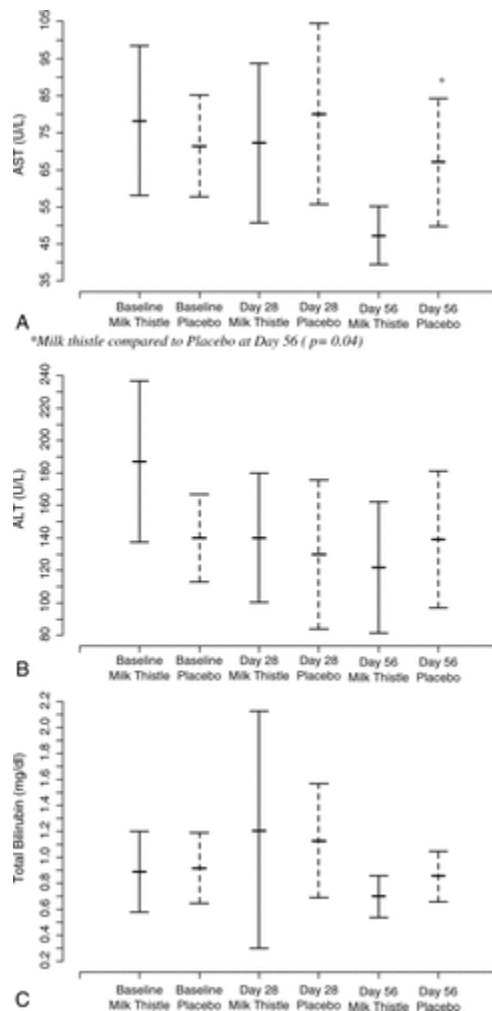


Figure 3. (A) Mean changes in AST and 95% confidence intervals at each time point by randomized group are presented. *Milk thistle compared with Placebo at Day 56 ($P = .04$). (B) Mean changes in ALT and 95% confidence intervals at each time point by randomized group are shown. (C) Mean changes in TB and 95% confidence intervals at each time point by study arm are depicted.

We evaluated the mean reductions in intraindividual AST and ALT over time by using difference scores from baseline to Day 28 and from baseline to Day 56. No significant differences between MT and placebo in AST or ALT from baseline to Day 28 ($P = .55$; $P = .50$, respectively) were observed. At Day 56, the MT group had a significantly lower AST ($P = .05$) and a trend toward a significantly lower ALT ($P = .07$) from baseline than the placebo group.

We did not observe a significant difference in mean TB levels at each of the time points. However, at Day 28, 5 patients in the MT group and no patients in the placebo group had greater than a 50% reduction in total bilirubin during the intervention period ($P < .007$).

Administration of Chemotherapy

We investigated the effects of MT on median doses and reductions in doses of chemotherapy and delays in administration of chemotherapy. The median dose (range) of each chemotherapy agent that was potentially modifiable for hepatotoxicity and administered during the intervention period in the MT and placebo groups were as follows: Methotrexate, MT 25 mg/m²/week (10-75 mg/m²/week); Placebo, 20 mg/m²/week (10-40 mg/m²/week); 6 mercaptopurine, MT 500 mg/m²/week (75-1300 mg/m²/week); Placebo, 413 mg/m²/week (235-900 mg/m²/week); and Vincristine, MT 1.7 mg/dose (0.6-2 mg/dose); Placebo, 1.4 mg/dose (0.8-2 mg/dose). Four of 23 and 3 of 26 patients in the MT and placebo groups, respectively, experienced a delay in therapy. Fourteen (61%) of patients in the MT group and 19 (73%) of patients in the placebo group received a reduction in dose during the 4-week intervention period. No significant differences in doses of chemotherapy administered, reductions of chemotherapy doses, or delays in treatment between the 2 groups were observed.

Toxicities and Adherence

No significant differences in chemotherapy-related grade 3 or 4 toxicities were observed between the 2 groups (Table 2). Hematologic and infectious toxicities were observed in 6 patients in the MT group and in 17 patients in the placebo group. Nonhematologic toxicities were observed in 9 patients in the MT group and 6 patients in the placebo group. No significant differences in the number or severity of toxicities or rates of infection were found.

Table 2. Number of Patients by Group with Grade 3/4 Toxicities and Infections

	Hematologic	Neurological	Hepatic	Gastrointestinal	Infection
Days 0 to 28					
Milk Thistle	3	0	5	0	2
Placebo	4	1	3	0	0*
Days 28 to 56					
Milk Thistle	2	0	8	0	5
Placebo	6	0	5	0	8†

* Infections during Days 0 to Day 28 were pneumonia, upper respiratory infection.

† Infections during Days 28 to Day 56 were *Pneumocystis jirovecii* pneumonia, pneumonia, rotavirus, bacteremia, upper respiratory infection, otitis media, sinusitis.

The patient-reported side effects in the MT group were diarrhea,² flatulence,¹ irritability,² and stomach ache.² In the placebo group, patient-reported side effects were decreased appetite,¹ diarrhea,² stomach ache,² and soft stools.¹ The patient-reported side effects were mild and were pre-existing complaints before the initiation of treatment with milk thistle. No significant differences in patient-reported side effects were found between the 2 groups.

Adherence to the protocol was 68% for the MT group and 96% for the placebo group ($P = .02$). We observed a significant difference in age between patients that adhered to the study protocol (mean age, 6.9 ± 3 years) in comparison to patients with poor adherence (13.1 ± 5.4 years) ($P = .01$).

DISCUSSION

This is the first randomized, controlled, clinical study to investigate the feasibility and safety of the herbal plant, milk thistle, in combination with the administration of chemotherapy in children undergoing treatment for cancer. We found that a short course of MT can be administered to children in the maintenance phase of treatment for ALL. No unexpected toxicities, reductions in doses of chemotherapy, or delays in therapy were observed during the MT supplementation period, despite the intervention group receiving slightly higher doses of vincristine and 6 mercaptopurine and experiencing a lower percentage of chemotherapy dose reductions. Our preclinical data demonstrate that MT does not compromise the anticancer activity of L-asparaginase or vincristine in CCRF-CEM cell lines.

The administration of a 28-day course of MT was associated with a significant reduction in AST and a trend toward a significant reduction in ALT at Day 56 but not immediately after cessation of supplementation. The effect observed on AST and ALT could be due to delayed effects of milk thistle, inadequate dosing, or short duration of supplementation. An evaluation of clinical literature shows a wide range of therapeutic doses and duration.⁵ At the time of development of this clinical trial for supportive care, phase 1 studies were not routinely developed for investigation of herbal or nutritional supplements.^{18, 19} Therefore, we chose a short course of treatment and a conservative dose, as this was the first trial conducted among children undergoing treatment for cancer. A recent phase 1 study in men with prostate cancer suggested a dose of 13 g per day for future trials; thus our dose may have been too conservative.²⁰ Phase 1 trials are needed in our patient population to determine appropriate dose and duration for both prevention and treatment of hepatic toxicity.

Our study was strengthened by the product quality analysis, stability testing, and the goal of quantifying plasma levels of silibinin. Although detectable silibinin plasma concentrations were not observed at the doses prescribed, several hypotheses could account for this. Our limit of detection was 15 ng/mL (0.03 μ M) for each compound with a mean recovery of 52%. This corresponds to 0.06 μ M for each Silybin A or Silybin B, or 0.12 μ M for the silibinin mixture. Previous studies in adults who had been administered a similar dose found total silibinin plasma concentrations were quite variable and nearly undetectable (0.3 μ M \pm 0.3 μ M or 144 \pm 144 ng/mL).¹⁶ Because silibinin analysis is comprised of 2 compounds (Silybin A and B) that equate to roughly 72 ng/mL each, the detectable concentrations of each compound was approximately 36 ng/mL or within 2-fold of our limits of detection.

Hoh et al collected blood samples within 1-4 hours of the final dose.¹⁶ In the current study, we relied primarily on patient reporting on the timing of the last dose, and the time to blood draw was outside the 4-hour range in several cases. When operating near the limits of detection, the timing of blood draws may be particularly crucial and should be closely controlled in future studies.

Finally, no information is available on the metabolism of silibinin in pediatric patients compared with adults. Children may metabolize silibinin isomers at rates different from that of adults. Taken together, this combination of confounding variables contributes to the lack of Silybin A or Silybin B detection in patient plasma samples.

Our study was weakened by a small sample size. Based upon previously published studies, our study was powered to detect a reduction in liver function tests of 50% at minimum. However, analysis of the current data (data not shown), found that this reduction was only 30%, which was achieved according to AST levels at Day 56. Therefore, our current study was not sufficiently powered to detect a significant treatment effect at Day 28.

We also found that the MT group had a significantly lower compliance rate compared with the placebo group. We hypothesize that the treatment effect would be more pronounced had the compliance rate been improved in the MT group. Furthermore, assessment of liver toxicity by ALT and particularly AST levels are limited by their lack of specificity for chemotherapy-induced hepatocellular injury.

Despite our study's limitations, it provides preliminary evidence that MT may be a safe, effective, supportive-care agent. Future investigations are needed to determine the appropriate dose and duration and to identify populations that may gain the largest clinical benefit. Possible populations are those undergoing treatment for acute myelogenous leukemia or stem-cell transplantation in which hepatotoxicity frequently results in interruptions of treatment. Hepatoprotectant agents could also advance the management of patients with total parenteral nutrition-induced hepatotoxicity.

In conclusion, this was the first study to evaluate milk thistle, a commonly used dietary supplement, in a blinded controlled trial among children undergoing treatment for ALL with biochemical evidence of elevated liver function tests. Future clinical trials should explore MT in the setting of patients in which hepatic toxicity prevents provision of the recommended chemotherapy in individuals with cancer.

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CONFLICT OF INTEREST DISCLOSURES

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REFERENCES

- 1 Greenlee H, Abascal K, Yarnell E, et al. Clinical applications of Silybum marianum in oncology. *Integr Cancer Ther.* 2007; **6**: 158-165.
- 2 Sagar SM. Future directions for research on Silybum marianum for cancer patients. *Integr Cancer Ther.* 2007; **6**: 166-173.
- 3 Tamayo C, Diamond S. Review of clinical trials evaluating safety and efficacy of milk thistle (Silybum marianum [L.] Gaertn.). *Integr Cancer Ther.* 2007; **6**: 146-157.
- 4 Comelli MC, Mengs U, Schneider C, et al. Toward the definition of the mechanism of action of silymarin: activities related to cellular protection from toxic damage induced by chemotherapy. *Integr Cancer Ther.* 2007; **6**: 120-129.
- 5 Rambaldi A, Jacobs BP, Gluud C. Milk thistle for alcoholic and/or hepatitis B or C virus liver diseases. *Cochrane Database Syst Rev.* 2007; CD003620.
- 6 Invernizzi R, Bernuzzi S, Ciani D, et al. Silymarin during maintenance therapy of acute promyelocytic leukemia. *Haematologica.* 1993; **78**: 340-341.
- 7 Schmiegelow K. Prognostic significance of methotrexate and 6-mercaptopurine dosage during maintenance chemotherapy for childhood acute lymphoblastic leukemia. *Pediatr Hematol Oncol.* 1991; **8**: 301-312.
- 8 Farrow AC, Buchanan GR, Zwiener RJ, et al. Serum aminotransferase elevation during and following treatment of childhood acute lymphoblastic leukemia. *J Clin Oncol.* 1997; **15**: 1560-156.
- 9 Barzaghi N, Crema F, Gatti G, et al. Pharmacokinetic studies on IdB 1016, a silybin-phosphatidylcholine complex, in healthy human subjects. *Eur J Drug Metab Pharmacokinet.* 1990; **15**: 333-338.
- 10 Davis-Searles PR, Nakanishi Y, Kim NC, et al. Milk thistle and prostate cancer: differential effects of pure flavonolignans from Silybum marianum on antiproliferative end points in human prostate carcinoma cells. *Cancer Res.* 2005; **65**: 4448-4457.
- 11 Matloub Y, Lindemulder S, Gaynon PS, et al. Intrathecal triple therapy decreases central nervous system relapse but fails to improve event-free survival when compared with intrathecal methotrexate: results of the Children's Cancer Group (CCG) 1952 study for standard-risk acute lymphoblastic leukemia, reported by the Children's Oncology Group. *Blood.* 2006; **108**: 1165-1173.
- 12 Seibel NL, Steinherz PG, Sather HN, et al. Early postinduction intensification therapy improves survival for children and adolescents with high-risk acute lymphoblastic leukemia: a report from the Children's Oncology Group. *Blood.* 2008; **111**: 2548-2555.

- 13 National Cancer Institute Common Toxicity Criteria. Bethesda, Maryland: National Cancer Institute; 2009.
- 14 Ladas EJ, Kroll D, Kelly K. Milk thistle. In: Coates P, Levine M, Blackman M, Moss J, Cragg G, White J. *Encyclopedia of Dietary Supplements*, 1st ed. New York, New York: Marcel Dekker; 2004: 467-482.
- 15 Kim NC, Graf TN, Sparacino CM, et al. Complete isolation and characterization of silybins and isosilybins from milk thistle (*Silybum marianum*). *Org Biomol Chem*. 2003; **1**: 1684-1689.
- 16 Hoh C, Boocock D, Marczylo T, et al. Pilot study of oral silibinin, a putative chemopreventive agent, in colorectal cancer patients: silibinin levels in plasma, colorectum, and liver and their pharmacodynamic consequences. *Clin Cancer Res*. 2006; **12**: 2944-2950.
- 17 Chou TC, Talaly P. Analysis of combined drug effects: a new look at a very old problem. *Trends Pharmacol Sci*. 1983; **4**: 450-454.
- 18 Straus SE, Chesney MA. Science and government. Enhanced: in defense of NCCAM. *Science*. 2006; **313**: 303-304.
- 19 Parkman CA. The NCCAM and new research priorities. *Case Manager*. 2004; **15**: 28-32.
- 20 Flaig TW, Gustafson DL, Su LJ, et al. A phase I and pharmacokinetic study of silybin-phytosome in prostate cancer patients. *Invest New Drugs*. 2007; **25**: 139-146.
- 21 Malempati S, Gaynon PS, Sather H, La MK, Stork LC; Children's Oncology Group. Outcome after relapse among children with standard-risk acute lymphoblastic leukemia: Children's Oncology Group study CCG-1952. *J Clin Oncol*. 2007; **25**: 5800-5807.
- 22 Panosyan EH, Seibel NL, Martin-Aragon S, et al. Asparaginase antibody and asparaginase activity in children with higher-risk acute lymphoblastic leukemia: Children's Cancer Group Study CCG-1961. *J Pediatr Hematol Oncol*. 2004; **26**: 217-226.