Milk thistle nomenclature: why it matters in cancer research and pharmacokinetic studies

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Abstract:

Extracts of milk thistle have been recognized for centuries as "liver tonics" and are well-known to prevent or reverse hepatotoxicity of reactive drug metabolites or naturally occurring toxins. Milk thistle extracts are now under intense study in the experimental therapeutics of cancer for chemoprevention, treatment, and amelioration of chemotherapy side effects. Precision in nomenclature, however, has lagged behind this progress. The crude commercial product of milk thistle is termed silymarin, a complex of at least 7 flavonolignans and 1 flavonoid that comprises 65% to 80% of milk thistle extract. From silymarin is derived silibinin, a semipurified fraction once thought to be a single compound but now recognized as a 1:1 mixture of 2 diastereoisomers, silybin A and silybin B. The distinction between silymarin and silibinin is not only important to understanding the historical literature, but thorough characterization and use of chemically defined mixtures in preclinical and clinical studies are essential to the progress of these botanical compounds as human therapeutics. As a result, we urge clinicians and preclinical investigators alike to exercise rigor in nomenclature and use pure compounds or precisely defined chemical mixtures in subsequent studies. Herein, we provide a guide to the proper nomenclature and composition of milk thistle extracts and discuss the known pharmacokinetic studies of these botanical medicines. The drug-interaction potential of these extracts appears to be quite low, and in fact, silibinin appears to synergize with the antitumor effects of some commonly used chemotherapeutics. However, some precautions are advised as high-dose, phase II studies are conducted.

Keywords: milk thistle; silymarin; silibinin; flavonolignans; stereochemistry; cancer; epithelial

Article:

"The beginning of wisdom is to call things by their proper names."
Chinese proverb attributed often to Confucius

Milk thistle (Silybum marianum [L.] Gaertn. [Asteraceae]; synonym Carduus marianus L.) is a hardy and often invasive plant indigenous to the Mediterranean region whose achenes, often referred to as seeds, have been valued for their medicinal qualities. (1) Most research and application of the phytomedicinal extract of milk thistle seeds had been directed toward disorders of the liver, particularly cirrhosis, hepatitis, and in protection and treatment of xenobiotic-mediated liver injury. In the early 1990s, several reports began to appear suggestive of milk thistle as a potential cancer chemopreventive agent. The 1994 report of Agarwal et al (2,3) demonstrating silymarin inhibition of tumor promotion by phorbol ester in the SENCAR mouse is viewed widely as the catalyst for studying milk thistle as a cancer preventive and potential treatment. The subsequent progress of milk thistle in cancer research will be covered elsewhere in this volume by Deep and Agarwal. (4) The purpose of this review is instead to discuss the chemistry and composition of milk thistle extracts, discuss the pharmacokinetic studies with milk thistle extracts, and urge a renewed attention to detail in milk thistle nomenclature to clarify the literature and maximize the potential clinical applications of this promising botanical medicine.

Nomenclature of Milk Thistle Compounds

In the August 2000 issue of Hepatology, the prolific Czech consortium of milk thistle researchers led by Drs Vilim Simanek and Vladimir Kren emphatically raised attention to proper milk thistle nomenclature in a letter to the editor entitled, "Silymarin: What Is in the Name ...? An Appeal for a Change of Editorial Policy." (5) Their essay, in response to a review of herbal products for liver diseases, raised several key issues that converged on the inappropriate nomenclature and incomplete characterization of milk thistle products published in preclinical and clinical studies. The authors closed their editorial as follows:

> We strongly recommend that investigators should either (1) give the exact composition of the "silymarin complex" or "the principle" used, or (2) use pure, chemically defined compounds. Implementation of these changes would greatly clarify the literature describing the biology of silymarin and its constituents. (5)

This issue is not a new one in the study of botanical medicines, as most plant extracts contain a number of bioactive compounds yet are usually only standardized (if at all) for 1 compound or a number of compounds with similar spectroscopic characteristics. As milk thistle extracts prove increasingly useful in preclinical studies, progression to clinical studies will require complete and precise chemical characterization of study materials. As members of our research group were the first to purify milligram quantities of all 7 milk thistle flavonolignan compounds for biological studies, (6) we hope to answer the call of our colleagues in promoting clarity in the nomenclature of biologically active compounds derived from milk thistle.

Table 1 contains a quick glossary of terms used to describe milk thistle components in the literature, often interchangeably and, unfortunately, incorrectly. The term "silymarin," a clever condensation of the plant's Latin binomial, was introduced originally in 1968 by the revered German phytochemist, Professor Hildebert Wagner, and colleagues to describe the mixture of flavonolignans that had been characterized at the time (silybin, isosilybin, silydianin, and silychristin). (7,8) Although many studies were conducted over the ensuing 3 decades, the Research Triangle Institute Natural Products Laboratory first reported on the resolution and purification of all 7 flavonolignans from this mixture (Figure 1). (6) What was first termed silybin can be resolved into 2 diastereoisomers, silybin A and silybin B. A similar diastereoisomeric mixture exists, yielding isosilybin A and isosilybin B (which are regioisomers of silybin A and silybin B). The other 3 flavonolignans, which are "constitutional" or "structural" isomers of the aforementioned compounds, are silychristin, isosilychristin, and silydianin. The isolation and absolute stereochemistries of all these compounds were subsequently confirmed by Lee and Liu. (9) The chemical structures of the 7 flavonolignans and taxifolin are shown in Figure 1.

Figure 2 displays high-pressure liquid chromatography (HPLC) chromatograms of silymarin, silibinin, and isosilibinin mixtures relative to all of the compounds isolated individually (silybin A, silybin B, isosilibin A, and isosilybin B on the left panel and taxifolin, isosilychristin, silychristin, and silydianin on the right panel). For those readers who are not well-versed in the intricacies of chromatography, one can consider these chromatograms like fingerprints. It is our intention to demonstrate the remarkable differences between these substances, which are all too often treated in the literature as being equivalent. From this, it should be apparent that silymarin is a complex mixture, silibinin and isosilibinin are each a mixture of 2 compounds, and all of the compounds can be isolated from each other (see Table 1 for a quick guide to these terms).

Why is silymarin such a complex mixture? Silymarin is simply the initial extract of milk thistle seeds made with ethanol (or other solvents (10,11)) that contains 65% to 80% total flavonolignans and a small amount of a flavonoid (taxifolin, barely discernable in the HPLC chromatogram of silymarin) (Figure 2), with the remainder containing fatty acids and polyphenolic compounds. (1,6,12) The most commonly cited silymarin product used

in clinical trials is Legalon, or Thisilyn in the United States, a milk thistle extract sold by Madaus AG that is standardized to 80% silymarin. Hence, a 175-mg capsule of Thisilyn contains 140 mg of a mixture of the 8 compounds in Figure 1.

The most abundant compounds in silymarin are the 2 diastereoisomers that comprise the semipurified mixture, silibinin. One of the problems with silibinin reports in the literature is that it is considered a single compound when, in fact, it is 2: roughly equal percentages of silybin A and silybin B. Even Sigma-Aldrich, the source of silibinin for most preclinical studies, continues to sell its silibinin (#S0417) with a description that implies it is a purified single compound from silymarin.

Why the Nomenclature Matters Outside of Chemistry

First, and most obviously, any preclinical or clinical study should be informed by the knowledge of whether one is testing a mixture of 8 compounds or 2. Particularly in the case of silymarin, the abundance of each of the compounds may vary depending on the source of botanical material, supplier, and extraction processes employed.

Nature is variable, even within the same species of plant grown in the same exact place. This fact is well-accepted by those who enjoy wine, where a bottle created by the same vintners, grown on the same vines, but produced in 2 different years can be quite distinct. The same can be true of herbal materials. Yet, all too often in the scientific literature, many herbal extracts are treated, erroneously, as being completely equivalent. (13) In clinical studies of pharmaceuticals, GMP-grade material is used such that the identity and reproducibility of the study agent can be ensured. In clinical studies of botanicals, however, this is often not the case, making comparisons between trials nearly impossible.

Biologically, the relative importance of each compound is emerging as pure compounds are isolated from silymarin or silibinin in quantities sufficient for pharmacological evaluation. The importance of mixtures or pure compounds will likely depend on the biological endpoint under study and the intended therapeutic intervention. For example, the silymarin mixture has been shown to contain a modest ligand for the [beta] form estrogen receptor (ER[beta]). (14) ER[beta] expression may serve to prevent breast cancer growth and serve as an antagonist to the mitogenic effects of ER[alpha]. (15-18) Follow-up transcriptional activation studies have revealed that silybin B and taxifolin are the only compounds in silymarin capable of stimulating an estrogenresponsive reporter plasmid construct in T47D breast cancer cells. (19) Both are of similar potency ([EC.sub.25] of 4.4 and 11.0 [micro]M, respectively), but taxifolin achieved a higher level of maximal stimulation. (19) We have shown that 3 silymarin products range from 21.6% to 23.8% silybin B, whereas taxifolin is present only at 1.6% to 2.2%. (12) With regard to silymarin, silybin B may be most important for its ER[beta] activity, but the data also suggest that pure taxifolin might be investigated individually as a breast cancer chemopreventive.

In our work with prostate carcinoma cells in culture, (12) 4 compounds had the most consistent antiproliferative effects across LNCaP, DU145, and PC3 cells: silybin A, silybin B, isosilybin A, and isosilybin B. Of these, isosilybin B was uniformly the most potent compound, but isosilybin A was equally efficacious (DU145) in some cases, whereas silybin B was as efficacious in others (PC3). In the androgen-dependent LNCaP prostate carcinoma cell line, prostate-specific antigen (PSA) secretion was most effectively suppressed by isosilybin A and isosilybin B. (12) Therefore, a combination of these 2 compounds, which we term "isosilibinin," may be preferable for future studies in prostate carcinoma.

Conversely, the free radical scavenging activity of pure compounds is reported to vary considerably, with silydianin and silychristin exhibiting 2- to 10-fold greater potency than the silibinin mixture. (20) Silydianin and silychristin are present exclusively in silymarin; they are absent from silibinin. This factor likely accounts for

the observation that, on a mass basis, silymarin is 8-fold more potent than silibinin as a free radical scavenger. (20)

Therefore, as biological studies progress, it remains important to make the distinction between silymarin and silibinin, and their respective and distinct compositions. In fact, 1 herbal marketer has already cited our 2005 Cancer Research paper (12) and labels their silymarin product explicitly as containing isosilybin B, the most broadly potent of the flavonolignans in arresting prostate cancer growth (Mega Silymarin with Isosilybin B, Life Extension, Hollywood, Fla). This clever marketing, however, obscures the truth that all silymarin products we have tested contain some isosilybin B, albeit at concentrations at 2.1% to 4.4% by weight. (12) Other compounds are equally effective at higher concentrations, but this is not the case in all biological endpoints. Hence, a careful analysis of the biological activities of each pure compound should be undertaken in any system where either preparation exhibits promising activity.

The distinction between silymarin and silibinin is also important as clinical investigators might seek to pool existing studies for meta-analyses. Even silymarin products vary in chemical composition among themselves depending on the source of the milk thistle extract. So, there is some hesitation in even comparing results with silymarin products obtained from different suppliers, for example, as shown in the pharmacokinetic results of Schulz et al. (21) While silymarin does indeed contain a subset of the compounds in silibinin (silybin A and silybin B), we would further hesitate to pool silymarin and silibinin data, as the nonsilibinin compounds in silymarin may have distinct effects. Indeed, some of these effects may be overlapping and redundant, such as suppression of human prostate carcinoma cell proliferation in vitro. (12) But depending on the outcome monitored, it is entirely possible that silymarin and silibinin may behave differently. Therefore, in any consideration of the clinical responses to milk thistle extract, careful distinction should be made between silymarin and silibinin, at the very least.

A Few Words About Units of Concentration

There is 1 caveat as milk thistle studies are discussed: nearly all published pharmacokinetic studies use total silibinin as the measurement but treat it as a single pure compound, expressing its concentration in terms of molarity, usually micromolar ([micro]M). Semantically, molar values should be reserved for pure compounds. Concentrations of mixtures, like silibinin and silymarin, are usually referred to in the natural products literature in micrograms per milliliter ([micro]g/mL), particularly because mixtures often contain compounds with different molecular weights. Expression in molarity helps one compare potency of compounds that differ in their molecular (or formula) weight. In the case of silibinin, both compounds share the same molecular weight of 482.1, and in silymarin, 7 of the 8 primary compounds also share this same molecular weight.

Hence, a "30-[micro]M" solution of silibinin is actually a mixture of 15 [micro]M silybin A and 15 [micro]M silybin B because we know silibinin is roughly a 1:1 mixture of each compound. 12 However, in a pharmacokinetic study, a reported concentration of 30 [micro]M silibinin in plasma does not tell one the respective contributions of each compound, particularly because each compound can be handled differently by the body. This is not purely a semantic issue of chemistry because 1 study has demonstrated that after in vitro incubation with liver microsomes, silybin B is glucuronidated far more efficiently than is silybin A. (22) Therefore, it is highly likely that these 2 compounds have distinct pharmacokinetic characteristics after being administered orally as silibinin.

Now that analytical methods exist to separate the various milk thistle flavonolignans, (6,9,23) we encourage those conducting future pharmacokinetic studies to use the molarity term only for pure compounds. In discussions that follow, however, we will use the term "[micro]M silibinin equivalents" to refer to studies that treat silibinin as a single compound.

Pharmacokinetics of Milk Thistle Compounds--Low-Dose Studies

Clinical studies of milk thistle extracts in cancer have lagged behind preclinical studies, but there have been 2 very recent, repeated dose-escalation phase I pharmacokinetic studies (24,25) that have followed from several single- and repeated-low-dose studies published in the 1990s. These studies are essential in moving milk thistle extracts forward as potentially useful antitumor adjuncts for the simple and similar reason that many pharmaceutical candidates do not move forward to efficacy trials: they fail to achieve meaningful plasma or tissue concentrations. Therefore, a primary purpose of pharmacokinetic studies is to provide assurance and correlation that the dose administered gives rise to plasma and/or target tissue concentrations consistent with those required to produce effects in vitro. One fault of many clinical studies of botanicals is that adequate pharmacokinetic analyses are not completed prior to initiating efficacy trials. We have noted previously that some botanicals may fail in efficacy trials not because the botanical is itself without activity, but because the dosing was not sufficient to achieve pharmacologically meaningful concentrations. (26)

Pharmacokinetic studies of milk thistle extracts are particularly important in making in vitro-in vivo correlations because the flavonolignans are notorious for their poor and erratic bioavailability. A 1995 study (21) revealed that among 3 silymarin products standardized for similar silibinin content, total bioavailability of a single dose varied by 2.2-fold, and peak plasma concentrations varied by nearly 3-fold. Another study reported on 1 participant from a group of 9 whose peak plasma silibinin concentrations were 20-fold greater than the mean of the other 8. (27)

To maximize oral bioavailability, the formulation most commonly employed in pharmacokinetic trials has been a mixture of silibinin and phosphatidylcholine sold by Indena SpA (Milan, Italy) as silipide, Siliphos[R], or IdB 1016. Barzaghi et al (27) reported on a trial in normal healthy volunteers given 120 mg silibinin equivalents bid for 8 days. Mean peak plasma concentrations at day 1 and day 8 were 240 ng/mL and 183 ng/mL, corresponding to 0.50 and 0.38 [micro]M, respectively. Terminal half-life ranged from 2.6 to 3.4 hours. This early study noted that the flavonolignans were highly conjugated to glucuronic acid with under 3% of the total dose recovered in the urine.

Weyhenmeyer et al (28) sought to identify the disposition of the 2 silibinin diastereoisomers but only referred to them as isomer 1 and isomer 2 owing to the lack of reference standards at the time (we now surmise these to be silybin A and silybin B, respectively) (Figure 2). In this study, 6 healthy male volunteers were given single doses of a silymarin product (Legalon 140, Madaus AG, Koln, Germany) standardized to 51 mg silibinin equivalents per capsule in a 4- way change-over design for dose escalation (102, 153, 203, and 254 mg silibinin). The bioavailability and peak plasma concentrations of isomer 1 were 2- to 3-fold greater than that of isomer 2, and mean peak plasma concentrations of combined isomers ranged from 0.24 [micro]M (at the 102-mg dose) to 0.66 [micro]M (at the 254-mg dose) silibinin equivalents.

Schandalik et al (29) examined biliary secretion of flavonolignans because the liver is the drug target for traditional uses of the herb in mushroom poisoning. These investigators examined both silipide and silymarin (source not defined), both dosed at 120 mg total silibinin. Remarkably, peak concentrations of silibinin equivalents achieved a mean of 116 [micro]g/mL (240 [micro]M) in the bile after silipide and 29 [micro]g/mL (60 [micro]M) after silymarin. Hence, biliary concentrations were 250 to 1000 times plasma concentrations reported previously. This study was also notable for examining other flavonolignans in bile. The low-abundance isosilybin diastereoisomer mixture appeared in bile at nearly the same concentrations as silibinin, despite the former being present in silymarin at one tenth the concentrations of silibinin. Unfortunately, the plasma determinations in this study were of low sensitivity such that even silibinin was only detectable in 2 of 9 patients. Nevertheless, this study is often cited as evidence for the 4.2-fold preferential bioavailability of silibinin from silipide versus silymarin.

As this last study was completed about the time of the first milk thistle chemoprevention paper from the Agarwal group, (2) one must consider the concentrations of silibinin required in vitro to observe the tumor cell

growth-arresting effects in the interpretation of past and current pharmacokinetic studies. In studies of various epithelial cancer cell lines, double-digit micromolar concentrations of silibinin equivalents have usually been required to observe changes in most endpoints. The secretion of IGFBP-3 by prostate carcinoma cells occurs with concentrations as little as 2 [micro]M, (30) but the [IC.sub.50] of silibinin equivalents for growth inhibition or apoptosis is usually greater than 20 [micro]M in most studies. (12,31) In 2002, Singh et al (32) conducted the most complete pharmacokinetic and pharmacodynamic studies in athymic nude mice harboring DU145 human prostate carcinoma xenografts. Two treatment groups were employed in this study with animals given silibinin in the diet at 0.05 and 0.1% (w/w). Monitoring of feed intake revealed that the mean dose in each group was 65 and 130 mg/kg per day, respectively. After 60 days, tumor volume was reduced by 35% and 58%, with tumor wet weights reduced by 29% and 40%, respectively. IGFBP-3 in plasma increased 4- and 5.8-fold, respectively, providing a pharmacodynamic confirmation of in vitro findings. Most important, however, were the plasma and tissue concentrations associated with these responses. Plasma levels of silibinin equivalents were 7 to 13 [micro]g/mL (15-27 [micro]M silibinin equivalents), and prostate concentrations were 3.7 to 4.6 [micro]g/g tissue wet weight (6-10 [micro]moles silibinin equivalents/g wet weight). (32) Therefore, these preclinical studies guided the design of subsequent human pharmacokinetic studies in aiming to achieve plasma or tissue concentrations greater than 10 to 15 [micro]M silibinin equivalents. It was clear that dosing far in excess of conventional dietary supplement label indications would be required to obtain these levels.

In more recent human studies, Hoh et al (24) tested somewhat higher conventional doses (up to 1.44 g/d) of silibinin-phosphatidylcholine (silipide; IdB 1016, Indena SpA) in 12 patients who were to undergo colorectal resection and another 12 who were to undergo liver surgery for hepatic metastases of colorectal cancer. The implicit but not stated rationale was that the high biliary concentrations observed in previous studies might be equally significant in liver and intestinal mucosa. Three oral dose levels (360, 720, and 1440 mg silibinin daily) were selected for 7 days prior to surgery, and blood levels of parent compound and metabolites were measured, as well as the concentrations in colorectal and hepatic tissue. Although analytical methods showed 2 chromatographic peaks for silibinin, the authors unfortunately did not differentiate between silybin A and silybin B in their quantitative measurements. Mean plasma concentrations of 3 to 4 [micro]M were achieved in the highest dose group. In the liver group, hepatic tissue concentrations were quite variable and ranged from 1.0 to 2.5 [micro]M across the dosage groups. Hence, older studies show that considerable enrichment of silibinin in the bile (29) is not associated with similar increases in hepatic parenchyma. Instead, the colorectal mucosal levels were dramatically higher than blood concentrations (141 [+ or -] 169 [micro]M at the highest dose level) but also quite variable. The investigators also examined pharmacodynamic endpoints including IGF-1, IGFBP-3, and the lipid peroxidationgenerated DNA adduct, [M.sub.1]dG, but none of these were altered significantly during this 7-day trial.

Pharmacokinetics of Milk Thistle Compounds--High-Dose Studies

Flaig et al (25) recently provided the best evidence that silibinin can be administered to humans at doses producing anticancer-relevant concentrations with minimal or no side effects. This study employed the largest doses to date ranging from 2.5 to 20 g of silibinin-phosphatidylcholine (Indena's Siliphos brand "silybinphytosome") daily given in 3 divided doses for 4 weeks to 13 men with a history of prostate carcinoma (mean age of 70 years). To accommodate these large doses, Siliphos powder was mixed with apple sauce at a ratio of 1/4 teaspoon powder to 1 tablespoon apple sauce. A dose of 5 to 10 g/d was required to achieve mean peak plasma concentrations above 25 [micro]M silibinin equivalents. However, escalation to 15 to 20 g/d did not increase concentrations above 50 [micro]M silibinin equivalents and was discontinued due to asymptomatic hyperbilirubinemia, due most likely to inhibition of the glucuronyltransferase, UGT1A1. When the largest cohort of 6 patients was given a daily dose of 13 g/d, mean peak plasma concentrations of 75 [micro]M silibinin equivalents were obtained. Mild hyperbilirubinemia was still observed but improved with treatment cessation in all patients.

The half-life of plasma silibinin ranged from 1.8 to 5 hours, consistent with previous studies, and there was extensive glucuronidation. Urinary free silibinin and conjugates were subject to a high degree of interpatient

variability. Hence, a clear dose response was not discernible: mean silibinin in the urine was 6.4 [micro]M silibinin equivalents (range, undetectable to 28.2 [micro]M), and mean silibinin-glucuronide was 253 [micro]M silibinin equivalents (range, 1.5-982 [micro]M silibinin). Although the main objective of the trial was to recommend a dose for phase II trials, PSA was followed in all patients, but none exhibited greater than a 50% decrease in PSA levels. However, several patients exhibited prolonged stable disease.

These data are the first to suggest that a milk thistle extract administered to human volunteers can be achieved in plasma at concentrations of silibinin equivalents consistent with inhibition of prostate carcinoma cell growth in culture. (25) However, these studies also point to a very important consideration if silibinin is to be used in the oncological setting. The authors report that the hyperbilirubinemia observed was due to inhibition of UGT1A1, whose [IC.sub.50] has been reported at 1.4 [micro]M silibinin equivalents. (33) Glucuronidation of the semisynthetic camptothecin derivative, irinotecan, is quite sensitive to UGT1A1 status, and patients with a UGT1A1 polymorphism that impairs activity are very sensitive to irinotecan toxicity. (34,35) A previous study indicated that milk thistle extracts do not influence irinotecan pharmacokinetics, but plasma silibinin equivalents achieved then were no greater than 0.26 [micro]M silibinin, below the [IC.sub.50] for UGT1A1 inhibition. Therefore, high doses of silibinin (>13 g/d) should be used cautiously in patients with compromised hepatic function and should be contraindicated for concomitant administration with irinotecan.

One Final Word About Hepatic Effects of Milk Thistle Extracts

The potential for herb-drug interactions between milk thistle extracts and conventional pharmaceuticals appears to be quite low, (36) particularly at doses less than 1 to 5 g/d. However, it is recognized that several reports have appeared suggesting that silibinin or silymarin can inhibit some isoforms of the cytochrome P450 family, including CYP3A4 and CYP2C9. (33,37,38) In cancer therapeutics, these findings are important because several conventional chemotherapeutic drugs are also metabolized by CYP3A4, raising some concerns regarding other milk thistle-drug interactions in oncology or other settings. Inhibition of purified enzymes was somewhat substrate selective, with [K.sub.i] values for silibinin equivalents of 32 to 166 [micro]M for CYP3A4 and 5 [micro]M for CYP2C9. (33) In cultured human hepatocytes, CYP3A4 activity was inhibited by 50% at 100 [micro]M and 100% at 250 [micro]M silymarin, concentrations that are quite high and difficult to relate to silibinin equivalents. (38) It bears noting that Gurley et al (36) reported that silibinin administration to human volunteers had no effect on model substrate metabolism by any of the 4 CYP isoforms his group measured, including CYP3A4; however, the dose used likely would not have produced plasma concentrations greater than 1 [micro]M silibinin equivalents.

There are several reasons to note the potential for CYP inhibition by milk thistle extracts as clinical efficacy trials might move forward. First is that plasma concentrations of silibinin equivalents at an oral dose of 13 g/d (25) may begin to approach concentrations for CYP inhibition. Second is that Sridar et al (33) noted that in vitro enzyme inhibition was "mechanism-based," meaning that inhibited P450 activities could not be restored by dialysis of silibinin and that inhibition may have resulted from a reactive metabolic intermediate that bound covalently to the heme moiety of the enzymes. This irreversible type of inhibition would pose a potentially more serious drug interaction risk if it were to occur in human hepatocytes or, obviously, human volunteers. However, there is no information at this point to suggest that P450 inhibition occurs in patients, and there has yet to be a conclusive demonstration of a covalent silibinin-P450 heme adduct in any in vitro study. In fact, there has yet to be a systematic dissection of cytochrome P450 inhibition by any of the single, pure flavonolignans. Hence, even if clinically relevant P450 inhibition were observed, it may only be restricted to individual flavonolignans. In this theoretical case, offending compounds could be separated from those that retain anticancer activity.

In the meantime, clinicians should be aware that high doses of milk thistle extracts, perhaps greater than 5 g/d, may have the potential to inhibit the metabolism of other drugs that are substrates for CYP3A4 or CYP2C9. Investigators conducting future high-dose studies may also wish to consider investigating the disposition of

CYP model substrates to address this important question, particularly since there is a growing appreciation for the potential metabolic interactions between drugs and dietary substances. (13)

Conclusions

The primary intent of this review article has been to make a strong case for accuracy in the description of milk thistle study materials used in in vitro, in vivo, and clinical studies. The argument has been made that attention to proper nomenclature is not germane only to chemistry but rather is essential to the further progress of milk thistle studies in the preclinical and clinical experimental therapeutics of cancer. Without attention to this important detail, comparisons between studies are fraught with errors. The authors recognize that FDA regulations for approval of a botanical investigational new drug application will likely dictate that milk thistle extracts, rather than pure compounds, will continue to move forward in clinical trials. Therefore, it is essential that all involved in basic and translational cancer research recognize the chemical complexity of the botanical extracts being tested and that greater attention be paid to the pharmacodynamic and pharmacokinetic characteristics of the pure compounds that comprise these promising therapeutic mixtures.

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Table 1. Quick Reference Glossary of Milk Thistle Nomenclature

Milk thistle extract	The initial extract of crushed milk thistle seeds, usually with ethanol, that contains 65% to 80% silymarin and 20% to 35% fatty acids, such as linoleic acid.
Silymarin	A complex of at least 7 flavonolignans and 1 flavonoid that comprises 65% to 80% of milk thistle extract.
Silibinin	A semipurified, commercially available fraction of silymarin. Silibinin was once thought to be a single compound and is often treated so in the literature. In fact, silibinin is a roughly 1:1 mixture of 2 diastereoisomeric compounds, silybin A and silybin B.
Isosilibinin	Similar to silibinin, this semipurified fraction of silymarin contains a roughly 1:1 mixture of 2 diastereoisomeric compounds, isosilybin A and isosilybin B. Isosilibinin is currently under evaluation as a more potent alternative to silibinin that may be produced more economically than either pure compound.
Flavonolignan	The most common class of compound present in milk thistle extract. Milk thistle flavonolignans result from a peroxidase reaction in the plant that fuses the flavonoid, taxifolin (known also as dihydroquercetin), with coniferyl alcohol, resulting in at least 7 compounds that all share the formula weight of 482.1.