Digestive Diseases Interagency Coordinating Committee
Liver Disease Subcommittee

Silymarin as Therapy of Liver Disease

A Workshop Sponsored by the

National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK),
National Center for Complementary and Alternative Medicine (NCCAM), and
National Institute on Alcohol Abuse and Alcoholism (NIAAA)
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Two Democracy Plaza
6707 Democracy Boulevard
Bethesda, Maryland

Welcome
Stephen E. Straus, M.D.
Director, NCCAM, NIH, Bethesda, MD

Dr. Jay H. Hoofnagle (Chief, Liver Disease Research Branch, Division of Digestive Diseases and Nutrition, NIDDK, National Institutes of Health (NIH)) opened the meeting and introduced Dr. Stephen E. Straus. Dr. Straus described the partnership between NIDDK, NCCAM, and NIAAA as an evolving process of seeking opportunities for the exploration of common complementary and alternative practices used by the American public. As a result of that partnership, those involved have determined that the most effective means of investigation in this area will require a step-wise approach, including the selection of preclinical, Phase I and II studies, and optimal animal models, which will be used as a scientific basis for the justification of larger randomized control trials. To that end, Dr. Straus expressed his confidence that the diversity of expertise present at the workshop would offer much needed input and guidance to the committee.

Session 1: Preclinical Studies of Silymarin
Standardization of Silymarin
David Lee, Ph.D., NPI, Inc., Belmont, MA

Milk thistle is one of the oldest and most researched herbal remedies in the world. In the last 50 years, the bioactive ingredients of the milk thistle have been identified, the major activity being ascribed to silymarin, a collection of flavinoids with antioxidant potential. Results of clinical studies of milk thistle have been controversial, in part because of the use of non-standardized products and dosages that are not well justified. Nonetheless, the excellent safety record of milk thistle makes it a product which merits further research and evaluation.

The objective of the Small Business Inovative Research (SBIR) Grant awarded to Dr. Lee was "to develop a standardized milk thistle product and to assure a quality controlled and reliable source for future basic and clinical studies." Specifically, the work was aimed at:

- Botanical identification and cultivation;
- Extraction and isolation of marker compounds;
- Standardization;
- In vitro and in vivo evaluations;
- Bioavailability and pharmacokinetic studies;
- Toxicity study;
- Formulation;
- Preparation of a drug master file (DMF); and
- IND application.

Milk thistle unlike many herbals exists as only two species: *Silybum mariabum* (with pink flowers) and *Silybum marianum var. albiflorum* (with white flowers). Milk thistle is extracted from the seeds of an annual plant. Varying the timing of collection results in dramatic differences in bioactive compound composition.

Silymarin is being prepared under this SBIR grant in a production facility in China. Compressed silymarin seeds yield oil which has long been used by local people as cooking oil, and more recently, has been shown to have potent antioxidant properties. A 90% acetone solution was used as a solvent to extract silymarin, since acetone has the advantage of being more inexpensive (approximately half the cost of ethyl acetate) and water soluble, making it easier to recycle. Further evaporation of remaining acetone yields a residue that subsequently is rinsed with hexane. The resulting precipitate is then washed with a 1% sodium chloride solution; the silymarin is collected, washed a second time, combined and blended to produce the final silymarin preparation.

To standardize the silymarin product, the individual components and stereoisomers were isolated and identified by high pressure liquid chromatography (HPLC). Major components include silybin A and B, isosilybin A and B, cis-silybin A and B, and neusilychristin. Which of these components is the "active" ingredient of milk thistle remains unclear. An *in vitro* cytoprotective assay was developed to assess the various silymarin isolates. The viability of cultured mouse hepatocytes was assessed after a 24-hour treatment with hydrogen peroxide using cystal violet. All isolates had activity and some of the minor components such as cis-silybin-B had the highest bioactivity.

HPLC analysis was also used to assess 12 different commercially available milk thistle products. All 12 had at least trace amounts of all the silymarin components. Other contaminants were present in most products as well. Overall traces were similar in the 12 products but there were differences in ratios of the individual components.

Features of the final silymarin product (MK-001) can be summarized:

- All components existing at at least 1% have been identified, characterized and evaluated.
- The extraction procedure is highly cost effective and is conducted under Good Manufacturing Practices (GMP) conditions.
- MK-001 is free of insecticides, pesticides, and heavy metal contamination.
- The final product follows FDA pharmaceutical standards.
- An IND has been submitted.
- MK-001 will be available for Phase I, II and III studies and the company will assure a reliable source, highest quality, and GMP packaging.

Discussion

Moderators: Sam Zakhari, Ph.D., Director, Division of Basic Research, NIAAA, NIH and Jose Serrano, M.D., Ph.D., Program Director, Liver, Biliary, and Pancreas Programs, NIDDK, NIH, Bethesda, MD

Dr. Sam Zakhari (Director, Division of Basic Research, NIAAA, NIH) asked how the compounds used in the in vitro studies compared in activity to standard antioxidants, such as vitamin E. Dr. Lee responded that no side-by-side comparisons have been made as these are preliminary data and further work needs to be done using different assays and conditions.

Dr. Serrano asked whether there were differences between the two milk thistle plants, the pink versus the white flowered plant and how timing of harvest was standardized. Dr. Lee stated that his group has focused solely on seeds from the pink flowered herbal and that harvesting at different seasons resulted only in changes of total concentration of silymarin in the seeds but that the components remain the same.

Dr. Mark Zern (Professor, Internal Medicine/Transplantation, University of California, Davis) raised the issue of the incidental removal of beneficial components from the seeds through the purification process. Dr. Lee agreed that this is an area for further study, albeit a difficult one due to the high water solubility of those minor components.

Dr. Hoofnagle commented that the HPLC results suggested that the majority of milk thistle products on the market are fairly well patterned. Dr. Lee agreed but stated that a few products have considerable impurities. Dr. Hoofnagle asked whether they intended to produce a highly purified silybin A for clinical use. Dr. Lee stated that they were not developing a product with a single silymarin component, but believed that a final product will contain all of the major and minor components in their natural ratios.

Dr. Strauss expressed interest in the issue of extracting, purifying, and characterizing individual constituents, and emphasized the importance of understanding which components are active and whether they synergize or compete with each other in some way, both in terms of efficacy and toxicity. Milk thistle is so well characterized and so devoid of other constituents that it may well provide an ideal setting to determine whether the compound as a whole is advantageous. Dr. Lee added that subsequent studies would be conducted to compare the standard with individual products.

Antioxidants and Liver Disease

*Craig J. McClain, M.D., Professor, Division of Gastroenterology/Hepatology, Vice
Chair for Research, University of Louisville, Louisville, KY*

Oxidative stress is defined as the imbalance between endogenous, intraccellular prooxidants and antioxidants. Oxidative stress is a frequent finding in liver disease which suggests that antioxidant therapy may be of value. The major reactive oxygen species include superoxide anion, hydrogen peroxide, hydroxyl radical; the reactive nitrogen species are nitric oxide (NO), and peroxynitrite (ONOO-); and reactive halide species are the hypohalous acids (e.g., HOCl). These molecules are important in oxidative stress either directly or as messengers or signaling molecules. Endogenous sources of reactive oxygen species include mitochondrial dysfunction in alcoholic liver disease, nonalcoholic steatohepatitis (NASH), and, to a lesser extent, hepatitis C. Transition metals (such as copper and iron), myeloperoxidase from infiltrating neutrophils, and increased CYP2E1 are also important in diseases such as alcoholic hepatitis and NASH. Markers used to assess reactive oxygen species and their damage, including biomarkers of DNA oxidation and antioxidant defense systems, are potentially clinically useful in assessing the severity of liver disease and need for treatment.

A study using a methionine-restricted, choline-deficient rat or mouse model showed that animals placed on the diet for 4 weeks developed fibrosis, cirrhosis, stellate cell activation, increased TBARS, and increased 4HNE, results which indicate a progression of simple fatty liver to fibrosis and ultimately cirrhosis, with some cases ultimately developing liver cancer (Chawla et al. Am J Physiol 275:G125-G129). Many of these changes can be prevented by pretreatment with antioxidants including S-adenysl methionine (SAMe), vitamin E and procysteine.

In studies of normal volunteers fed alcohol, markers of lipid peroxidation appeared in the serum (Meagher et al. J Clin Invest 1999; 104:805-13). Similarly, patients with alcoholic hepatitis have elevated markers of lipid peroxidation compared to controls. Stable alcoholic cirrhotics and patients with chronic hepatitis C have elevations in these markers as well. Patients with NASH also had footprints of oxidative stress as shown by peroxinitrate staining of hepatocytes in patients with fatty liver and NASH compared to individuals with normal livers (Sanyal et al. Gastro 2001;120:1183-92).

In an effort to examine the effectiveness of antioxidant intervention for the down-regulation of oxidative stress or pro-inflammatory cytokines, stable alcoholic cirrhotics were treated with procysteine. Glutathione levels rose and pheripheral blood monocyte production of pro-inflammatory cytokines such as IL-8 and TNF was decreased (Pena et al. JPEN 1999;23:1-6). These results, combined with studies cited earlier, provide a rationale for utilizing antioxidant therapy, especially since there are biomarkers that can be assayed in alcoholic liver disease. At present, a large multicenter trial of SAMe in alcoholic liver disease is about to be intiated funded by the Department of Veterans Affairs.

Several studies have suggested a role of antioxidant therapy for NASH. An open label trial of vitamin E in children demonstrated consistent improvements in serum aminotransferase levels (Lavine J. J Ped 2000:136;734-8). A 6-month period of diet followed by the addition of vitamin E for a year was shown to result in improvements in ALT and TGFβ1 levels as well as liver histology (Hasegawa et al. Aliment Pharmacol Ther 15:2001;1667-72). Finally, a lifestyle modification and vitamin E study in which 14

patients with biopsy-proven NASH were randomized to diet and exercise, with or without vitamin E, showed that the interventions resulted in decreased liver enzymes and markedly improved hyaluronic acid levels (a marker for fibrosis and sinusoidal endothelial cell dysfunction) over control individuals (Kugelmas et al. Hepatology 2003; 38:413-9). None of these studies were fully controlled or of adequate size and duration to prove the efficacy of antioxidant therapy of NASH. Two multicenter, large-scale randomized controlled trials of vitamin E versus insulin-sensitizing agents have been developed by the NASH Clinical Research Network which may help define the role of antioxidant therapy of nonalcoholic fatty liver disease.

Thus, oxidative stress plays an etiologic role in liver injury and fibrosis. While antioxidants are promising interventions, the types, combinations, dosing, and targeting remain important questions to be answered.

Discussion

Dr. Stephen Straus (NCCAM) remarked that the diseases under discussion could cause injury other than oxidative stress to the liver, and that the benefits of milk thistle, if any, may be due to effects on those other injuries. If solely an oxidant is needed, Dr. Strauss suggested that there may be much more potent, cost effective, and simpler antioxidants than milk thistle suitable for study. Dr. McClain agreed and recommended using animal models or in vitro studies to compare milk thistle to vitamin E or SAMe.

Dr. John Senior (FDA) asked whether the methionine-restricted diet model of fatty liver disease was reliable in causing chronic injury and fibrosis. Dr. McClain responded that methionine-restricted animals do not lose weight, but by 2 weeks have severe fatty liver, and by 4 weeks have fibrosis. After 3 months, most animals have cirrhosis. The model occasionally fails because there is too much methionine in the regular diet, which must be carefully controlled to produce the desired results.

Silymarin and Cell Injury
Mark Zern, M.D., Professor, Internal Medicine/Transplant, University of California Davis, Sacramento, CA

Milk thistle earned its common name from the milky veins in its leaves. Extracts of milk thistle have been reported to protect liver cells in vitro from a wide variety of toxins, including acetaminophen, ethanol, carbon tetrachloride, galactosamine, and iron, as well as ischemic injury, radiation, and viral hepatitis. Strong evidence from both human studies and animal studies in mice and dogs suggests that silymarin and its components are effective in countering *Amanita phalloides* mushroom poisoning. Furthermore, several controlled trials of silymarin have suggested that it has a beneficial effect in alcoholic liver disease and cirrhosis. The mechanism of action of silymarin has not been proven, in vitro and in vivo evidence indicating that it has distinct antioxidant, antifibrogenic, anti-inflammatory, and other hepatoprotective actions.

The major effects of silymarin are believed to be mediated through the antioxidant actions of this flavinoid. In large numbers of studies, silymarin has been shown to decrease oxidative injury from a variety of sources, inhibiting lipid peroxidation and membrane damage. Several studies have suggested that silymarin inhibits activation of

NF-κB, a selective effect in that the drug does not affect AP1 activation. Silymarin blocks the activation of NF-κB by phorbol ester, lipopolysaccharide, okadaic acid, and ceramide, but not from H_2O_2. It appears to inhibit phosphorylation and degradation of IκB, and inhibits the translocation of p65 to the nucleus. Silymarin also inhibits TNF-α-induced activation of MAP kinase and its activation of caspases (Manna et al, J Immunol 1999;163:6800). In a study in mice, silybin inhibited the oxidative CYP 450 enzymes 1A1, 1A2 which may limit hepatotoxicity through inhibition of activation to reactive intermediates (Baer-Dubowska et al, Xenobiotica 1998; 28: 735).

In humans, silybinin has been shown to have oxygen radical scavenging effects and to inhibit the arachidonic acid pathway (Dehmlow et al, Life Sciences 1996;58:1591). The most significant effect observed was inhibition of perchlorate release by neutrophils, but silybinin also appeared to interfere directly with 5-lipoxygenase activity. Silybinin also had inhibitory effects on PG2 production, which may also be hepatoprotective. Human studies in patients with alcoholic cirrhosis, indicate that silymarin decreases lymphocyte expression of superoxidase dismutase (Feher et al, Acta Med Hung 1988;45:265).

Silymarin has been shown in a variety of different studies to affect liver cell injury by toxins by pathways other than antioxidant effects. Silymarin blocks the uptake of phalloidin by hepatocytes and may affect other transmembrane transport systems. The regulatory action of silymarin on cellular and mitochondrial membrane permeability associated with increased membrane stability against xenobiotic injury is supported by a number of studies. (Munter et al, Biochem Biophy Acta 1986;860:91). In a rat model of ischemia/reperfusion injury, silymarin reversed the severe impairment of mitochondrial bioenergetics, including increasing ATP levels, decreasing susceptibility to mitochondrial permeability transition (MPT), and improving defects in mitochondrial respiration (Rolo et al, Hepatol Research 2003;26:217).

Silymarin may also have direct effects on fibrogenesis. In a mouse model of biliary fibrosis without significant inflammation, silymarin inhibited collagen accumulation, even when administered late, inhibiting increases in serum PIIINP levels (Boigk et al, Hepatology 1997;26:643). Silymarin downregulated type 1 collagen, TIMP-1 and TGF-β1 mRNA levels in treated animals (Jia et al, J Hepatol 2001;35:392), suggesting that it has an effect on collagen-related genes which is separate from its antioxidant effect. Lieber and colleagues showed improvement in a 3-year study in alcohol-fed baboons, including decreases in serum ALT and 4-hydroxynoneal levels and suppresion of type 1 procollagen mRNA levels in liver (J Clin Gastroent 2003;4:336). Evaluation of the relative antifibrogenic and antioxidant effects of silymarin in humans warrants further and more careful investigation.

Silymarin has also been reported to have anti-inflammatory actions. Purta et al. demonstrated decreased edema and neutrophil infiltration in mice treated with silymarin following xylene (ear) or carrageenan (paw or peritoneal) administration. (J Pharm Pharmacol 1995;48:968). In rat Kupffer cells, silybinin decreased superoxide anion radical formation and production of nitric oxide and leukotriene B4 (Dehmlow et al, Hepatology 1996;23:749). Two studies from Hungary have suggested that improvement in immune function is a mechanism for beneficial effects of silymarin in alcoholic liver disease in humans. Silymarin reduced lectin-dependent and natural killer cell-mediated cytotoxicity by lymphocytes, but not antigen-dependent cytotoxicity (Deak et al, Orv

Hetil 1990;131:1291, and Lang et al, Tokai, J Exp Clin Med; 1990; 15:123). Concurrent with the normalization of ALT, AST and bilirubin levels in patients with alcoholic liver disease receiving silymarin, there were decreases in the percentage of OKT8+ cells and lymphocyte toxicity. In a more recent study, Schuman et al (J Hepatol 2003;39:333) showed that silybinin abrogated ConA-induced liver disease in mice by inhibiting TNF-α, IFN-γ, IL-2, IL-4, NF-κB, and iNOS, while augmenting IL-10 expression, which again, may be more of an immunomodulatory effect than has been shown in other studies.

A recent study of patients with alcoholic cirrhosis who were treated with silymarin MZ 80 for 6 months had statistically significant increases in total GSH and decreases in MDA, as well as decreases in PIIINP (Lucena et al, Int J Clin Pharm Ther 2002;40:2). However, not all studies showed improvement of oxidative stress with silymarin therapy. Halim and coworkers (Ann Clin Biochem 1997;34:656) showed a marked decrease in liver enzymes, and improvement in histology and survival with silymarin administration to CCl$_4$-treated rats, but no significant change in MDA levels, suggesting an improvement in perhaps a mechanism other than antioxidant effects.

Silymarin was shown to reduce the ethanol- or acetaminophen-enhanced, CYP2E1-mediated cytotoxicity of methotrexate in human hepatocytes, but at a relatively high dose (Neuman et al, Clin Biochem 1999;32:519), although other studies have not shown an effect on CYP2E1. In another study, liver microsomes from human donors showed inhibition of CYP3A4 and CYP2C9 activity with silybinin (Bechmann-Knopp et al, Pharmacol Toxicol 2000;86:250).

Thus the potential mechanisms of action of silymarin include:
- Antioxidant properties and the inhibition of lipid peroxidation; and
- Inhibition of NF-κB activation.
- Inhibition of fibrosis;
- Inhibition of inflammation;
- Enhancement of RNA, DNA, and protein synthesis;
- Regulation of cell permeability;
- Inhibition of mitochondrial injury;
- Immunomodulation; and
- Inhibition of P450 activity.

Discussion

Dr. Kris Kowdley (University of Washington) stressed the importance of using surrogate markers for actions of silymarin in clinical trials. Thus, use of serum, lymphyocyte and urine markers for oxidative stress, fibrosis, cytokine production and immune function are important during clinical studies seeking to elucidate the efficacy of milk thistle in liver disease. Serum markers of fibrosis, while not proven to be highly sensitive or specific, can be used to help identify the mechanism of action of silymarin and assess its activity in individual patients. Similarly, studies of lymphocyte subsets and function during therapy may help identify the dose and regimen most appropriate in humans. Thus, while ALT, AST and GGTP are commonly used as surrogate markers for improvement in liver disease, markers of fibrosis, oxidative stress and immune function

can supplement these tests while the ultimate criteria for benefit needs to be clinical (death, transplantation, decompensation) and histological.

Dr. Stephen Straus (NCCAM) suggested that animal models should provide some direction to use of indirect surrogate markers for the actions of silymarin and for changes in liver disease activity and grade.

Dr. Jay Hoofnagle (NIDDK) remarked that studies of silymarin suggested that it had multiple effects on cells that are potentially protective against injury and asked why the focus has been liver disease and not other forms of injury. Why also was it considered a superior antioxidant for liver disease than others such as vitamin E, selenium and SAMe.

Dr. Josh Berman (NCCAM) remarked that his Center will soon engage in an investigation of the relative antioxidant strengths of many natural products, with an aim to determine whether or not a certain natural product has potential as either a specific or more general antioxidant. He hypothesized that the most likely reason for the use of milk thistle in liver disease is a combination of inherent antioxidant actions and other more liver specific activities. These issues should be resolved in animal models, but ultimately, researchers will have to recognize the realistic limits of the pace of preclinical investigation versus the desire to help patients and initiate clinical trials early.

Dr. Straus (NCCAM) added that having a sense of at least the top three or four most reasonable mechanisms and the ability to discern at which time the product has the strongest activity allows one to begin to consider a dosing schedule to optimize the effects. He also pointed out that it is probably fairly optimistic to assume that researchers can identify a single effect; rather, it might be more beneficial to separate the major effect from more minor ones, and there are model systems available to do so.

Dr. David Lee (NPI) commented that most herbal remedies have multiple actions and targets. Nevertheless, it is convenient to focus on a single mechanism of action as this helps to guide clinical studies and to determine appropriate dose-regimens.

Dr. John Senior (FDA) commented that it was important to distinguish effects on models of acute versus chronic liver disease. The major interest in milk thistle has been on therapy of chronic liver diseases and cell injury. Important endpoints are prevention of fibrosis and clinical deterioration. Use of these endpoints requires large studies conducted over many years. A reasonable surrogate would be to focus on serious acute liver injury. Amelioration of acute injury has been demonstrated in animal models and deserves to be studied in humans as well.

Dr. Jay Hoofnagle (NIDDK) added that an important first step is to identify the appropriate dose of silymarin and this is best done using short-term surrogate markers for activity. Long-term outcome is the most appropriate endpoint for ultimate decisions on the benefit of silymarin therapy, but intermediate term endpoints or surrogate markers are needed to evaluate what dose needs to be given at what intervals for how long in which patients. Dr. Hoofnagle asked the cell biologists in attendance to suggest what surrogate markers would be best to evaluate in patients receiving silymarin. Drs. Craig McClain and Mark Zern both recommended use of lymphocyte levels of NF-κB or markers of oxidative injury. They also recommended use of a simple animal model to develop these markers and stressed the need to use normal volunteers in early studies of changes of these markers in humans given silymarin.

Dr. Kowdley stated that although the points raised are very relevant, silymarin is quite different from other new drugs under development. Millions of people are already taking this product, believing that it will help their liver disease. Thus, the usual concerns involved in the step-wise drug development process should be abbreviated. There is a certain urgency to demonstrating whether silymarin has clinical utility in liver disease. Thus, clinical studies should not be slowed for the sake of identifying all of the mechanisms of action of the drug and their relative importance.

Session 2: Clinical Studies of Silymarin
Clinical Pharmacokinetics of Silymarin

John Markowitz, Pharm. D., Associate Professor, Department of Pharmaceutical Sciences, Laboratory of Drug Disposition and Pharmacogenetics, Medical University of South Carolina, Charleston, SC

Silymarin is a polyphenolic flavonoid mixture isolated from the seeds of milk thistle *(Silybum marianum)*, composed mainly of silibinin (or silybin), which is thought to be the primary bioactive component, and lesser amounts of silydianin and silychristin (all silibinin stereoisomers). In addition, silibin itself consists of two diastereomers, silibinin A and B. Most studies have measurerd only the total amount of silibin A and B, rather than each diasteromer separately. In describing drug disposition, it is important to discuss liberation (an important aspect for a complex herbal preparation), absorption, distribution, metabolism, and excretion.

Silibinin is poorly absorbed and efforts to improve its bioavailability have been only partially successful. In both animal models and humans, the bioavailability of silibinin ranges from 20% to 50% following oral administration. Chemical complexation with phosphatidylcholine (forming *silipide*) greatly increases the lipophilic properties of silibinin and hence improves its bioavailability.

Oral administration of silipide formulations result in plasma silibinin concentrations that are several-fold higher than those achieved with standard silibinin (i.e., the mixture of any number of these constituents) or silymarin preparations in equimolar dosages (i.e., those doses that ideally have the same amount of silibinin) and overall enhanced bioavailability. Absorption is fairly rapid, with peak plasma concentrations (C_{max}) reached in approximately 2 to 4 hours (T_{max}).

There is little information on plasma or total body distribution of silymarin or extent of protein binding. The volume of distribution (Vd) in humans is also largely unknown. However, based on approximations from recently published pharmacokinetic studies, Vd may be relatively small (~0.6 L/kg). This relatively small number suggests that silibinin is limited to peripheral vascular circulation (plasma). In a study conducted by Zhao and Agarwal, silibinin was shown to be widely distributed in both free and conjugated forms in plasma and tissue, including liver, lung, stomach, skin, prostate, and skin following oral administration in the mouse (Carcinogenesis 1999;20:2101-8).

Little is known regarding the biotransformation of silibinin in humans beyond its conjugation. The majority of silibinin is conjugated and excreted in the bile, with only 3 to 8% of the parent (free) compound excreted unchanged. There is some evidence of

enterophepatic recycling (i.e., intestinal absorption, conjugation in the liver, excretion in the bile, hydrolysis of conjugates, and reuptake in the intestine).

One of the main issues surrounding pharmacokinetics of silymarin products is that the overwhelming majority of silibinin is conjugated, to either a glucuronide or sulfate conjugate. During the past decade or so, conjugates have been largely considered to be the molecular form that drugs are converted to for excretion. However, conjugated molecules can be active as well (e.g., morphine), and can be even more potent than the parent compound. The possibility of drug interactions has been suggested by the findings that silymarin may inhibit activities of CYP3A4 and UGT1A6/9 (glucuronide transferases), and P-glycoprotein (a drug afflux transporter) (Venkataramanan et al, Drug Metab Dispos 2000;28:1270-3; Beckman-Knopp et al, Pharmacol & Toxicol 2000;86:250-6; Zhang & Morris, J Pharmacol Exp Ther 2003;304:1258-67; Sridar et al., Drug Metab Dispos 2004;32:1587-94). However, an *in vivo* study performed in normal volunteers detected no interaction between silymarin and the CYP3A4/P-gp substrate indinavir (a protease inhibitor which is both a cytochrome P450 3A4 substrate and a PGP substrate) (DiCenzo et al, Pharmacotherapy 2003;23:866-70).

Silymarin is primarily excreted in urine as a conjugated metabolite, with significant biliary excretion as well. Half-life ($t_{1/2}$) of elimination is 3 to 4 hours, suggesting multiple daily doses of most oral formulations would be required. Biliary excretion can occur for sustained periods (up to 24 hours) following single doses.

In a recent three-way crossover study with a 7-day wash-out period between phases, the comparative bioavailability of three commercial formulations of silibinin was examined in 24 normal male volunteers. Each subject received a single oral dose equivalent to 120mg of silibinin on three separate occasions, using Liverman, Legalon, or generic silymarin capsules. Results indicated that Legalon and silymarin capsules performed similarly, while the Liverman capsules produced a slightly better profile, although the reasons for this were not clear. (Kim et al, Int J Clin Pharmacol & Ther 2003;41:593-6)

Most herbal products are composed of multiple constituents beyond their purported ingredients, prompting the need for understanding whole supplements, rather than individual constituents. Some herbal products may contain constituents which are potent inhibitors of a given isozyme; yet in totality no clinical effects are observed when the extract is consumed.

In vitro methods for screening for potential herb-drug interactions have the advantage of being high through-put, non-invasive (with no risk to human subjects), and in principle, can be used to forecast the magnitude of drug interactions. Several potential disadvantages, include:

- pharmacokinetics are not well characterized for most constituents;
- inability to accurately predict concentration at the intracellular site (problematic for herb/drug interactions and synthetic drug interactions);
- many constituents found in single herbal products may not reach appreciable concentrations;
- does not account for contribution of non-hepatic elimination, hepatic blood flow, protein and tissue binding, or possible contribution of other metabolites; and
- assessing metabolic *induction* is possible but more difficult.

Probe drug methodology is often used in clinical studies assessing drug interactions of both synthetic and herbal drugs. Probe studies do not provide information on clinical effect of a particular drug, but do use marker substrates that can be used to make broader generalizations about drug interactions. These studies are important in early evaluation of safety of new herbal products.

Discussion
Moderators: Margaret Chesney, NCCAM, Bethesda, MD

Leonard Seeff, MD, Special Expert on Viral Hepatitis, Liver Disease Research Branch, Division of Digestive Diseases and Nutrition, NIDDK, NIH, Bethesda, MD

Dr. Josh Berman (NCCAM) asked whether or not compounds such as silymarin that are heavily metabolized in the liver will show greater effects on liver disease than on other organs. Dr. Markowitz replied that flavinoids are often concentrated in the liver. Dr. Berman followed up with a question regarding acceptable levels of bioavailability of silymarin, to which Dr. Markowitz responded that even drugs with poor bioavailability may be effective if given in appropriate (higher) oral doses to compensate for lower bioavailability. However, without knowing what blood concentrations are necessary for a therapeutic effect, acceptable bioavailability levels cannot be determined.

Dr. Hoofnagle stated that since silymarin is fat soluble, a bioavailability of 30 to 50% is not surprising. He asked whether the mechanism of absorption had been identified. Dr. Markowitz answered that the drug is probably taken up by diffusion rather than being actively transported into cells, but that this was speculative.

Dr. Hoofnagle pointed out that, since the liver often activates drugs, this may be a clue that the conjugates may be more active than the native species of the compound. Dr. Senior suggested that because silymarin appears to inhibits CYP 3A4 activity, it may also be a substrate for 3A4. Given that it is largely unknown whether silymarin changes the metabolism of other drugs or whether those drugs alter the metabolism of silymarin, this area is important for further study.

Silymarin as Treatment of Hepatitis C

Kris Kowdley, MD, University of Washington, Seattle, WA

Use of complementary and alternative medications (CAM) is frequent among patients with chronic liver diseases including hepatitis C. In surveys of patients attending liver disease clinics, an average of 40% of patients admit to use of CAM and 15% use of herbal products, the most frequent of which is milk thistle or silymarin. Most patients take silymarin on their own initiative without professional medical advice. Unfortunately, there is often poor documentation of the ingredients of commercial silymarin products that are used and little information on its efficacy in hepatitis C or chronic liver disease.

There are a number of reasons why silymarin might be useful in the treatment of liver disease. Silymarin has antioxidant properties and influences both free radical-

mediated cytotoxicity and lipid peroxidation in *in vitro* and *in vivo* models of liver disease. Silymarin may also have antifibriotic effects.

Silymarin has activity against iron-related liver injury. In a study of carbonyl iron-fed rats, silybin significantly reduced production of malondialdehyde-protein adducts and improved mitochondrial energy efficiency and ATP stores (Pietrangelo et al. Gastroenterology 1995:109:1941-9). Silybin has multiple potential beneficial effects in liver injury, including reduction of lipid peroxidation, scavenging of free radicals, and prevention of iron accumulation. In addition, silybin may restore glutathione levels, which may have a significant impact on iron-mediated fibrogenesis and oxidative stress.

The effects of silymarin on hepatic fibrosis have been studied in a gerbil model of iron overload. Animals were given intramuscular iron dextran and either placebo or silybin. There were no differences in hepatic iron accumulation, however silybin treated animals had less hepatic fibrosis as shown by collagen staining. Hepatocytes also had reduced staining for heme oxygenase suggested reduced oxidative stress (Pietrangelo et al. J Bioenergetics and Biomembranes 2002;34:67-69)

In large scale meta-analyses of silymarin as therapy of liver disease, there was a slight trend towards improved serum ALT in the silymarin-treated group but a less difference in serum AST levels and no differences in albumin levels (Jacobs et al, Am J Med 2003:13-506-15). There was a trend towards improvement in prothrombin time levels in patients treated with silymarin.

The problem with interpreting clinical studies of silymarin is that few studies have focused on a homogenous patient population with a specific, well defined liver disease. Perhaps as a consequence, studies have had varying results. In an exhaustive search of both electronic and manual resources, the Cochrane review identified only 14 randomized trials that could be considered useful for evaluating the effectiveness of silymarin therapy for hepatitis C (Liu et al, Am J Gastroenterol 2001:98:538-44). This review concluded that there is no evidence for benefit of silymarin therapy.

A series of small clinical trials have been conducted using a standardized silymarin product - IdB1016 - which has a standardized concentration of silybin and has undergone extensive pre-clinical anlaysis. In a study of 10 patients with chronic hepatitis C who had failed to respond to a course of interferon-alfa, silymarin was given in a dose equivalent to 360 mg per day of silybinin for 2 months, followed by a 1-month washout and 2-month placebo treatment period. The placebo group was treated in inverse sequence. Compared to the placebo-treated patients, the 10 silymarin treated subjects had an 18% reduction in serum ALT, a 17% reduction in serum AST (which approached statistical significance), but no change in serum bilirubin and alkaline phosphatase levels. Similar results were observed in a pilot study in patients with chronic hepatitis given 120 mg of silybin equivalence twice daily (Moscarella et al, Curr Therap Res 1993; 53: 98-102).

In a subsequent Phase II placebo-controlled, 3-month study, 31 patients with chronic hepatitis were given 120 mg silybinin and 34 patients were treated with placebo twice daily for 3 months. IdB1016 treatment was associated with a significant decrease in both serum ALT and AST levels (Marcelli et al, Eur Bull Drug Res 1992; 1:131-5).

In an uncontrolled, dose-escalation study, patients with hepatitis C and alcohol-induced liver disease had significant reductions in serum ALT, AST and GGTP levels (Vailati et al. Fitoterapia, 1993. 64:219-28).

In a short term, randomized controlled study in chronic hepatitis C, 20 patients were given 240 mg silybinin daily (IdB1016) or placebo. Compared to placebo, there were signficant decreases in serum bilirubin, GGT, ALT, and AST levels within 7 days of starting therapy with silybinin (Buzzelli et al, Int J Clin Pharmacol Ther Toxicol 1993;31:56-60).

Supported by a small grant from the NCCAM, the University of Washington has designed a phase I/II study of IdB1016 in patients with chronic hepatitis C. A total of 36 patients will be stratified on the basis of histological stage (fibrosis scores of 2, 3 or 4) and randomized into one of 3 different doses of drug, ranging from 360 to 1890 mg of silymarin three times daily for 12 weeks followed by a 12-week observation period. Endpoints of therapy include tolerance and serum ALT levels with secondary endpoints being plasma TGF-β1, serum hyaluronic acid (HA), procollagen III peptide (PIIIP), YKL-40 (chondrex), ferritin, and iron saturation levels. Compliance, adverse effects, and health-related quality of life will also be measured. The study has received IRB approval, an IND is in place, and the first patients have been enrolled. No results are available.

Discussion

In response to questions from Drs. Berman (NCCAM) and McClain (Univ Louisville), Dr. Kowdley stated that the study is not placebo-controlled because it was considered a dose-finding trial and focuses on safety and evaluation of surrogate markers. In addition, patients receive a fixed dose and sequential cohorts are given higher doses.

Dr. John Senior (FDA) recommended that, if the pilot study yields adequate results leading to a larger randomized study, investigators ought to consider other measures of liver function (e.g., INR, direct and total bilirubin). Dr. Kowdley agreed, stating that the next step would involve a randomized, placebo-controlled trial with liver biopsy as an endpoint, looking at progression vs regression or stabilization of fibrosis.

Dr. Leonard Seeff (NIDDK) asked for clarification regarding the status of previous interferon therapy in the patients with chronic hepatitis C who would be enrolled in these studies. Dr. Kowdley explained enrolled patients will have received interferon in the past or have contraindications to interferon therapy. Dr. Kowdley suggested that the beneficial effect of silybin should be independent of responsiveness to the antiviral effects of interferon.

Silymarin as Therapy of Non-alcoholic Steatohepatitis (NASH)
Paul Angulo, Mayo Clinic

NASH is a common and frequently silent liver disease marked by fat accumulation in the liver along with inflammation, hepatocellular injury and fibrosis. Patients typically have few symptoms but can have mild to moderate elevations in serum ALT levels and evidence of fat in the liver by ultrasound. A liver biopsy is required to diagnose NASH, separating simple hepatic steatosis (fatty liver) from steatohepatitis. NASH resembles alcoholic liver disease histologically but by definition occurs in persons who drink little or no alcohol. NASH is a potentially progressive liver disease, and a

proportion of patients eventually develop cirrhosis, end-stage liver disease and, in some instances, hepatocellular carcinoma.

The etiology of NASH is only partially understood. The disease occurs largely in persons who are overweight or obese, particularly if there is concurrent type 2 diabetes and/or hyperlipidemia. Only a small proportion of people with normal body mass index (BMI of 20 to 25) have either simple hepatic steatosis or NASH. In contrast, more than 60% of obese persons have hepatic steatosis, and approximately 20% have NASH. In patients with severe obesity (BMI above 40), more than 80% have hepatic steatosis and almost half have some degree of NASH. With the rising rates of obesity and diabetes in America, NASH has become the most common cause of abnormal liver tests found in otherwise asymptomatic individuals.

The pathogenesis of NASH is not completely understood, but the most well accepted hypothesis postulates that the disease is caused by "two-hits" – the first causing steatosis and the second leading to injury and fibrosis. Insulin resistance is likely to account for the first hit in most patients, while oxidative stress and lipid peroxidation or proinflammatory cytokine injury is believed to account for the second.

There is no therapy of proven long-term benefit for NASH. Achieving and maintaining appropriate control of body weight appears to lead to improvement in NASH histologically. Weight control, however, is a difficult task to accomplish by most overweight or obese individuals. For patients with diabetes or hyperlipidemia, glucose and lipid level control are recommended, but do not always lead to improvements in liver tests associated with NASH. Finally, some patients with NASH are not overweight and do not have diabetes or hyperlipidemia. These considerations have led to the search for pharmacological therapies for this disease.

Several medications have been proposed as therapies for NASH. These medications include hepatoprotective agents such as ursodiol (ursodeoxycholic acid, UDCA), antioxidants such as vitamin E and betaine and anti-diabetic medications that inprove insulin resistance such as metformin and the thiazolidinediones (TZDs). Ursodiol is the only medication that has been evaluated in a adequately-sized, prospective randomized controlled trial (Lindor et al. Hepatology 2004;39:770). While ursodiol therapy was associated with mild improvements in serum aminotransferases and hepatic steatosis, similar improvements occurred in the control group and there was no improvement in hepatic inflammatory activity or fibrosis. Other medications have been evaluated in small, uncontrolled clinical trials with some evidence of benefit. The most promising results have been reported with the TZDs pioglitazone (Promrat et al. Hepatology 2004;39:188) and rosiglitazone (Neuschwander-Tetri et al. Hepatology 2003;38:1008) and antioxidants (Lavine et al. J Pediatr 2002;136:734; Hasegawa et al. Aliment Pharmacol Ther 2001;15:1667). Based on these results, two large, multicenter randomized controlled trials of vitamin E versus metformin or pioglitazone have been initiated recently under the auspices of the NASH Clinical Research Network.

Patients with NASH often have evidence of oxidative stress and lipid peroxidation suggesting that antioxidant therapy may be beneficial. Thus, in several clinical studies, patients with NASH were found to have significantly greater evidence of lipid peroxidation compared to patients with fatty liver alone and normal controls (Seki et al. J Hepatol 2002;37:56; Sanyal et al. Gastroenterology 2001;120:1183). Other evidence that oxidative stress plays a role in progression of NASH include findings of

malondialdehyde adducts (MDA) in the liver in patients with NASH which correlates with degree of fibrosis (McDonald et al. J Gastro Hepatol, 2001;16:599) and the finding that CYP2E1 is highly expressed in patients with NASH compared to individuals with steatosis alone and those with normal livers (Videla et al. Clinical Science 2004;61:2035).

Silymarin has not been studied prospectively and specifically in patients with NASH. Undoubtedly, some patients with cirrhosis due to NASH were included in the several studies of silymarin in patients with cirrhosis (Saller et al. Drugs 2001;61:2035). Those studies have demonstrated some evidence of benefit of silymarin although meta-analyses and Cochrane reviews have concluded that the overall evidence of benefit is lacking.

Investigators at the Mayo Clinic Foundation have designed a phase II, open-label pilot study of silymarin in patients with NASH.   Thirty patients with liver biopsy-proven NASH will be treated for 2 years with 600 mg silymarin daily, a dosage level chosen because it has been proven safe in patients with liver disease (Velssi et al. J Hepatol 1997;26:871), and some animal models have shown that higher dosages may produce greater antioxidant and antifibrotic effects (Boigk et al. Hepatology 1987;26:643). Patients will be followed at 3 month intervals with clinical evaluation and routine blood tests for serum aminotransferase levels. Serum markers of lipid peroxidation, including thiobarbituric acid reacting substances (TBARS) and oxidized LDL (ox-LDL), and markers of liver fibrosis (aminoterminal procollagen III peptide, collagen IV, and hyaluronic acid) will be measured annually. Liver biopsies will be taken at baseline and again after 2 years of treatment. At present, however, the study is on hold because the pharmaceutical company that prepares the silymarin has not been willing to provide the drug for clinical trials.

Discussion

Dr. Josh Berman (NCCAM) asked whether liver biopsies were necessary as an endpoint for efficacy. Dr. Jay Hoofnagle (NIDDK) responded that histological confirmation of benefit is required in evaluating many liver diseases and particularly in NASH. There is a poor correlation between the height of serum aminotransferase elevations and the severity of the underlying liver disease in NASH that makes liver biopsies necessary to demonstrate clinical benefit. Serum aminotransferase levels and other blood test results might be adequate surrogate endpoints for phase I studies assessing tolerance and dose regimens and may be adequate in phase II studies when assessing benign drugs such as silymarin. However, for definitive phase III studies, liver biopsies will be necessary to demonstrate meaningful clinical benefit. To date, imaging studies that measure hepatic fat have not been shown to be reliable enough to use as a clinical endpoint in studies of NASH, but ultimately may be very helpful.

Dr. Kris Kowdley (U Washington, Seattle) commented that liver biopsies are complicated by sampling variability and reproducibility, and suggested that the algorithms using markers currently being developed by commercial and research laboratories may, in the future, be useful for the prognostication of patients or as a surrogate for liver biopsy. Dr. Angulo remarked that one problem with serum markers of fibrosis in NASH is determining a standard to be used for the comparison of serum marker accuracy.

Approaches to Development of Silymarin as Therapy of Liver Disease. General Discussion
Moderators: Jay Hoofnagle & Josh Berman

Preclinical/Clinical Trials and Study Design

Dr. Josh Berman reviewed the steps needed for drug development. Typically, drug development is separated into three distinct steps: the actual manufacturing of drug, preclinical testing, and clinical trials. Dr. David Lee had discussed the manufacturing of a silymarin preparation from its cultivation, harvesting, purification and formulation. Preclinical testing has been done on many silymarin preparations, although not on the formulation prepared by NPI and perhaps not in a complete manner. If silymarin is a dietary supplement, preclinical testing is not legally required. However, crucial information may not be gathered if preclinical trials are not performed, leading to pivotal trials being conducted with less certainty and confidence.

The final step of clinical trials is also typically separated into three phases: phase I studies to establish the proper dose, dose-regimen, and safety usually using volunteers given single to multiple doses of the medication and monitored for drug levels, safety concerns and perhaps simple endpoints (such as ALT levels or cytokines). Phase II studies focus on developing a dose regimen and safety as well as providing some evidence for the degree of efficacy that might be identified. Phase III trials focus on demonstrating safety and efficacy and establishing firm medical evidence on which to base recommendations for use. Most clinical researchers only consider phase II and III studies, as phase I studies are usually done by pharmaceutical companies with contract clinical sites providing volunteers and carrying out the necessary evaluations. For an herbal such as silymarin, a major error is to circumvent the phase I and early phase II evaluation and proceed immediately to advanced phase II and phase III studies. These studies are expensive and require major investment of time and resources. Conducting phase III studies before the correct dose, dose-regimen and surrogaqte markers of efficacy are established can be very wasteful and result in negative results for a drug that is actually effective (when given in a different dosage or regimen).

For silymarin, perhaps only one phase III pivotal trial will be conducted, and it is important to define what disease or diseases should be included, how long patients should be treated and followed, and what endpoints should be used to judge efficacy. Thus, for the immediate development of silymarin as a therapy in chronic liver disease it is important to conduct the necessary groundwork of phase I and II studies and prepare for ultimately conducting a large, definitive trial in a well characterized cohort of patients for which there is at least some evidence that silymarin is effective. The three diseases that have been mentioned as possibly a target for silymarin therapy are alcoholic liver disease, nonalcoholic steatohepatitis (NASH) and chronic hepatitis C (not responsive to conventional antiviral therapy).

Dr. John Senior (FDA) stated that the easiest disease to study is NASH because of its frequency in the population and the current lack of any proven therapy of benefit.

Alcoholic liver disease is difficult to study because of the population and the importance of abstinence in recovery and outcome, a confounding variable that is hard to control. For hepatitis C, patients who have failed to respond to current regimens of peginterferon and ribavirin may represent a resistant group in whom other therapies may also be ineffective.

Dr. Mark Zern (UC Davis) stated that, within 3 or 4 years, protease inhibitors will be available for testing, at least for hepatitis C, which may be a significant concern in terms of designing the study, although not a reason to exclude hepatitis C as a study choice. Dr. Leonard Seeff (NIDDK) remarked that, of the diseases being considered, hepatitis C is currently the disease for which patient's self-administer silymarin.

Dr. Berman asked about the relevance of animal models in guiding decisions about which human disease to treat with silymarin. Dr. Craig McClain (U Louisville) indicated that the animal models of alcoholic and nonalcoholic steatohepatitis are fairly well established but that there are no adequate animal models for chronic hepatitis C. He suggested that silymarin might be evaluated in combination with peginterferon and ribavirin. Dr. Seeff indicated that silymarin may have an effect in ameliorating the side effects of peginterferon and ribavirin therapy in chronic hepatitis C and might well be studied for that indication.

Product Formulation and Registration

Dr. David Lee (NPI) emphasized that investigators are dealing with at least two silymarin products: the original mixture of purified silymarin and a water-soluble silybin derivative (which has enhanced bioavailability).

Dr. Berman asked whether or not registration of a NCCAM sponsored silymarin preparation required formulation similar to those from pharmaceutical or herbal medication companies (such as those produced by MADAUS and Indena), or whether it would be possible to register a product that does not have the massive preclinical data (such as the NPI formulation). Mr. Russ Fleischer (Senior Clinical Analyst, Division of Antiviral Drug Products, FDA) answered that his Division has received INDs for silymarin from several sponsors. There is guidance available on the development of botanical drug products which includes chemistry, manufacturing controls, and pharmacokinetics in humans. Products currently available in stores probably would not be candidates for registration because of their level of variability. Synthesized products would likely require a higher level of data to ensure adequate characterization. Dr. Senior added that the process used by the FDA for pure, single compounds is probably not appropriate for application in the use of herbal biological mixtures, and recommended further high-level discussion between the FDA and NCCAM. Dr. Senior affirmed the importance of product consistency and added that the FDA would also need data for ranges of human variability in absorbing the compound. Ground rules would need to be set for the IND formulation, and would include product composition, consistency, and stability. A second phase would involve data on human dose-tolerance levels, efficacy, no observed effect limits (NOELs), and dose-response levels. Controlled studies would be part of the Phase III effort.

Dr. Hoofnagle asked Dr. Senior for some specific procedures to be used in conducting appropriate maximum tolerated dose trials. Dr. Senior responded that no fixed rules for duration exist because that aspect of study design is drug-specific. Dr. Berman suggested that initial silimarin meetings should generate a solicitation for the drug product with which to conduct subsequent, 2-to-3 year, phase I and II studies on silymarin. This approach was used by NCCAM in developing a drug application to the FDA for cranberry. NCCAM AND NIDDK set 17 criteria, to which pharmaceutical firms responded with applications. Those criteria may be too stringent for the case at hand, and FDA and NIH should collaborate in choosing the criteria to form the basis for solicitation for silymarin to ensure it meets perception of what is needed for an NDA. The solicitation need not necessarily precede the Phase I and II human design; investigators interested in conducting either animal preclinical work or clinical work would be made aware that a product which follows a general set of solicitations will be made available at no cost.

Dr. McClain asked for clarification on whether or not the group was looking at making silymarin a new drug or whether they were looking at using doses that are similar to what have been used extensively in the past and proven to be safe.

Dr. Berman stated that NCCAM is considering registration of silymarin as a drug, not as a dietary supplement. He agreed with the earlier statement that the process should not require huge amounts of money. It should take a limited amount of funding for 3 years to conduct the appropriate phase I and II studies, and an as much as $25 million for the pivotal trial over the following 5 years. Dosing in phase I/II trials typically starts with the commonly used dose and, since the common dose it is unlikely to be as effective as desired, moves upward. Toxicity may become evident when non-presently used dosages are used. Phase I/II trials would utilize three endpoints: safety, surrogate markers for efficacy, and pharmacokinetics in terms of drug levels and total drug exposure as measured by areas under the curve (AUC).

Dr. Senior emphasized that the complex mixture of silymarin will likely prevent it from being developed as a standard drug, given FDA's rigorous requirements for proof of each component making a contribution with demonstrated evidence.

Dr. Hoofnagle pointed out that a dietary supplement only has to be proven to be pure, that it does not contain some contaminant. However, dietary supplements cannot claim to have efficacy in a disease. It seems that the nature of silymarin and its proposed use will require a special dispensation or statement from the FDA. Dr. Berman stated that cranberry is also a mixture, but NCCAM's approach is that they are using a biological as a drug and therefore they follow drug guidelines.

Dr. Senior explained that an IND is used to get approval for an indication of a new drug application. Using it for a claim requires an exemption from the law which says drugs or products cannot be marketed unless they have been proved to be safe and effective. What is at issue at this point is the possibility of "short-circuiting" that requirement for silymarin. Still, regardless of the fact that millions of people are already taking silymarin, its effects, especially at higher doses, need to be determined. Examples include recent experiences with germander and kava kava. Both agents had been used for centuries with no apparent toxicity, but both have been now found to be hepatotoxic when given in high doses.

Dr. Berman assured those present that drug impurities would not be a problem with silymarin; it will meet the standards and interpretations of the guidance and be available for investigators.

Mechanisms and Surrogate Markers

Dr. Berman recommended that both preclinical and small clinical investigations of mechanisms and surrogate markers ought to be conducted at the same time Phase I studies are being initiated, so that type of information is available in 3 or 4 years when the pivotal Phase III trials begin.

Dr. Hoofnagle raised the issue of which diseases ought to be investigated, since Phase I and II studies would need to be conducted in patients with disease, rather than just normal volunteers. Dr. Berman concurred, but again stated that proposals could be written for disease parameters that could be done preclinically and clinically prior to Phase III trials. Dr. Seeff agreed with Dr. Hoofnagle that determining which diseases ought to be studied is an important point, since markers may differ depending on which diseases are included. Dr. Angulo responded to a question from Dr. Hoofnagle regarding the surrogate markers he planned to use in his study of NASH. Dr. Angulo chose surrogate markers that are frequently elevated in patients with NASH, that are significantly higher in patients with NASH compared to levels in patients with simple steatosis and in normal controls. Dr. Kowdley also recommended that a critical first step ought to be to show some effect of silymarin on serum ALT, and to show it in comparison to a placebo.

Dr. Berman stated that manufacturing and toxicity are well defined because they are common for all products. However, how to relate those specifically to liver diseases may be limited because of the need to specify surrogate markers for efficacy.

Patient Selection and Compliance

Dr. Senior stated that studies need to be conducted with patients with liver disease, since the goal is to treat abnormalities and reduce them to normal as a result of a drug compared to placebo.

Dr. Markowitz commented that, once a product has been decided upon, both a pharmacokinetic study and basic safety laboratory monitoring (at baseline and follow-up) are easily incorporated into the same study. Studies need to be of sufficient size to examine variability in disposition of the active constituents, as well as variability between individual patients and genders, genetic influences, and so on. Many patients will be excluded from taking any other medications during the study, necessitating a fundamental Phase I pharmacokinetic study. It may be that enough commercial interest exists that drug companies may be receptive to initiating drug studies themselves, although one difficulty with CAM products is that there is little financial incentive for them to do so.

Dr. Senior asked whether there were significant numbers of people with chronic hepatitis C who are not eligible for the standard treatment to get meaningful results, since

that would eliminate the non-responder question. Dr. Seeff responded that, certainly, a large number of people do not fit the strict criteria for treatment, for a variety of reasons.

Dr. Kowdley added that several studies have shown that, in an urban VA population or a county hospital population, 10% or fewer of the patients with chronic hepatitis C are considered candidates for antiviral therapy, having significant liver disease but no contraindications.

Dr. Kowdley stated that data in clinical trials which select the best possible patients may claim response rates in genotype I patients of 50% or higher, but rates of response in practice are much lower. The numbers of patients with chronic hepatitis C are increasing, causing a burgeoning effect on the healthcare system. In Dr. Kowdley's experience, silymarin products are more frequently used by patients with hepatitis C than with NASH. From a biological point of view (the fundamental injury in NASH being due to oxidative stress), it may be attractive to evaluate silymarin in NASH, but there is currently a much more pressing need for an alternative therapy for hepatitis C. Dr. Zern agreed but also stressed that interferon non-responders are often more willing patients, because they have exhausted other therapies many of which are much more toxic.

Dr. Seeff brought up the issue of treating active drug users and those on methadone treatment, who may be potential study candidates. He also stated that compliance may be improved if the drug is administered by mouth and has few side effects. Dr. Hoofnagle stated that three-times-a-day pills may be a deterrent for compliance, and asked whether a once-a-day pill was a possibility. Dr. Markowitz answered that the products currently available are short-acting, and the drug has a relatively short half-life; however, it may be amenable to formulation like many other pharmaceutical drugs with even shorter half-lives. Dr. Berman remarked that twice-a-day would be preferable to three-times-a-day, irrespective of the pharmacokinetics, if it meant better compliance. In addition, pharmacokinetics may not be related to pharmacodynamics. Patients could experience three peaks of drug levels a day but still have only one peak of effect; thus, it may be that the effect on tissues is much longer than the 2 hours.

Dr. Markowitz emphasized that it is critical to remember that the active component is only bioavailable in very small amounts, and reminded participants that all the in vitro experimental work to date has been conducted essentially with a pure constituent in very low circulating concentrations.

Dr. Kowdley made the comment that investigators need to be aware of important changes in the community that might impact patient recruitment, such as private practitioners putting patients on indefinite, long-term interferon therapy. It is therefore important to expand the population beyond the non-responder population to include any individual that could not potentially be treated with interferon.

Endpoints

Dr. Berman commented on the difficulty in determining endpoints for drug treatment products and supported the idea of asking for a research solicitation requesting means of better defining endpoints of therapy.

In response to a question from Dr. Seeff, Dr. Hoofnagle stated that the NASH Clinical Research Network will be conducting a large multicenter trial, using nine centers. The study's efficacy endpoint will be histological improvements in the NASH activity index, a method for grading and staging of the liver biopsy, with secondary endpoints of quality of life, symptoms, and ALT levels. The group expects enzymes will improve with treatment. However, if enzymes improve without a change in histology, the biochemical improvements do not reliably reflect the severity of the underlying liver disease. In Phase I and early Phase II studies, endpoints might include ALT or a surrogate marker such as TBARS as guidance; investigators need not perform liver biopsies until there is some evidence of benefit. If ALT levels improve, however, it will be necessary to follow with a trial based upon liver histology to demonstrate that the changes are meaningful.

Dr. Seeff stated that the most important measure in assessing progression of liver disease is hepatic fibrosis. A critical need is to develop biomarkers that reliably reflect the amount of fibrosis or fibrogenesis. These biomarkers might ultimately replace liver biopsy as an endpoint for successful therapy. Similar comments might be made about imaging methods in assessing fibrosis and fatty change.

Dr. Seeff emphasized that the differences between hepatitis C and NASH will require different approaches to the problems at hand. Since hepatitis C patients cannot receive antiviral therapy concurrent with silymarin in these early studies, two groups might be studied separately: one receiving conventional treatment of peginterferon and ribavirin plus silymarin, and the other receiving silymarin alone, the latter group consisting of persons who have falied to respond to antiviral therapy or you have contraindications to its use. Drs. Hoofnagle and Zern answered that non-responders to interferon would be used, and that a decrease in enzymes would be realized, and perhaps a change over time in fibrosis, as well. While Dr. Seeff agreed that choosing individuals who have failed conventional therapy is probably acceptable at this point, the outcome would take years, and suggested investigators need to have a measure that indicates the dose is heading the patient in the right direction before investigators can make the decision to increase the dose.

Following the General Discussion Session, Dr. Hoofnagle thanked all those who participated, and adjourned the meeting.