Simple and Inexpensive Methods to Prevent Botanical Misfortune

By: David J. Kroll, Heather S. Shaw, James M. Mathews, and Nicholas H. Oberlies

Kroll, D.J., Shaw, H.S., Mathews, J.M. and Oberlies, N.H. (2006). Simple and inexpensive methods to prevent botanical misfortune. *Acta Horticulturae* 720, 137-147.

Abstract:

Botanical dietary supplements are often targeted by the mainstream media and scientific/medical communities as being unscientific and unsafe relative to prescription pharmaceuticals and over-the counter drugs. However, the relative risk of botanical medicines is largely overestimated and causal relationships between botanical remedies and significant adverse reactions pale in comparison to the frequency and magnitude of those observed with normal use of FDA-approved pharmaceuticals. Historically, a greater premium has been placed on demonstrating the safety of health-related products as opposed to confirming therapeutic efficacy; in fact, the required standards for prescription *drug efficacy* were implemented in the U.S. more than 50 years after standards for purity and safety were firmly in place. An argument will be made that botanical manufacturers can support their place in the national health agenda and generate significant goodwill by voluntarily subjecting supplements to relatively simple and inexpensive preclinical studies, and even limited phase I clinical trials, employing the basic tenets of pharmacology and toxicology (i.e., dose-response relationships) to support the relative safety of specific products. Moreover, many of the unfortunate adverse events of drug-herb interactions over the last 10 years could have been prevented by relatively simple hepatic metabolism and drug transport studies that have become commonplace in even the smallest of academic laboratories, due in large part to the increased availability and lower cost of previously scarce and *cumbersome* biological *reagents.* Demonstrating the safety of semi-pharmaceutical formulations of herbal products now requires more than simple reliance on the historic folk record of safe use; real health risks and negative publicity can be successfully mitigated with a modest, prospective investment in basic preclinical toxicology testing. A model is presented for a non-profit research center that focuses on botanical quality and safety as a first step toward this goal.
Keywords: adverse reactions, analytical chemistry, botanical safety, case reports, drug interactions, hepatic metabolism, herbal medicine.

Article:

INTRODUCTION

Since the explosive growth of the international botanical medicine industry in the 1990s, the market for this class of dietary supplements has leveled off. In the US and Europe in particular, there have been three primary causes for this dampening of enthusiasm. First, is the appearance of reports of significant drug-herb interactions in high-profile journals, whereby previously unanticipated effects of botanical components on inducing hepatic drug metabolizing enzymes resulted in subtherapeutic blood levels of drugs used to treat HIV/AIDS and organ transplant rejection (Moore et al., 2000; Piscitelli et al., 2000; Ruschitzka et al., 2000). Second, a number of reports surfaced on the contamination and/or adulteration of botanical products with toxic heavy metals and current or obsolete prescription drugs (Ernst, 2002; Levine et al., 2002; Pittler and Ernst, 2003; Saper et al., 2004). Third, and perhaps most damning to the industry, has been accumulation of clinical trials results indicating lack of therapeutic efficacy for some of the best-known, time-tested herbal remedies such as echinacea *(Echinacea* spp.)extracts for rhinoviral infections and saw palmetto *(Serenoa repens)* for benign prostatic hypertrophy (Turner et al., 2005; Bent et al., 2006).

We propose that each of these broad classes of botanical misfortune might have been prevented by modest industry investment in analytical and quantitative chemistry studies, some basic preclinical toxicology studies, and basic Phase I human pharmacokinetic trials to correlate botanical dosing with target plasma concentrations

of active constituents. In fact, one of the authors proposed such a coordinated approach at the 1997 Natural Products Expo East in Baltimore, MD, in his natural healing keynote address entitled, "Addressing the barriers to acceptance of herbs as medicines," to encourage the industry to use a scientific language and approach to cultivate botanical medicine use and acceptance among open-minded conventional healthcare practitioners (Kroll, 1997).

For example, botanicals can be easily studied for their effect on inhibiting drug- metabolizing enzymes in vitro or by inducing these enzymes in cultured human hepatocytes (LeCluyse et al., 2000; Mathews et al., 2002). Similarly, botanical supplements can be easily monitored for contamination or adulteration with heavy metals, prescription drugs, pesticides and herbicides, and microbes or mycotoxins (Levine et al., 2002; Lau et al., 2003). Lastly, clinical trial failures may be avoided by starting with an appropriately standardized and characterized botanical preparation and selection of doses capable of producing plasma concentrations of active principles consistent with biological efficacy surrogates observed in vitro (Kroll and Oberlies, 2003). Wolsko et al. (2005) recently addressed the problem of inadequate herbal supplement characterization in 81 published clinical trials. Only 12 (15%) of these trials reported performing tests to quantify botanical composition and 3 (4%) of the trials provided data to compare actual with expected content of at least one chemical constituent. The costs associated with these botanical quality analyses would likely have been lower than the lost sales the industry has experienced as a result. In addition, botanical characterization and pilot pharmacokinetic studies should have been considered as a wise investment by funding agencies before approving the execution of multimillion dollar randomized, placebo- controlled clinical trials.

As part of an independent, non-profit research institute, our research team is committed to the appropriate and safe access to effective botanical medicines by the general public. The methods, approaches, and philosophies are presented herein in the spirit of assisting our colleagues in the botanical industry of achieving this objective. The CFSAN-FDA International Conference on Quality and Safety Issues Related to Botanicals at Oxford, MS, USA, (2005) presented an opportunity to discuss, among a broad cross-section of stakeholders, the functional structure for an independent center dedicated to research on botanical quality and safety.

THE NEED FOR A RESEARCH CENTER FOR BOTANICAL QUALITY AND SAFETY

Botanical safety and herb-drug interactions represent two of the paramount challenges facing this country given the widespread use of herbal dietary supplements. Numerous statistics could be cited, and in particular, the 2004 NCCAM report on U.S. complementary and alternative medicine usage rates makes clear that tens of millions of Americans use at least one botanical supplement every year (Barnes et al., 2004). The very existence of the NIH-ODS Botanical Research Centers program illustrates that the existing federal safety and efficacy standards are disproportionate relative to the widespread use of botanicals. If botanicals were regulated in a manner similar to prescription or OTC drugs, where the burden falls on the manufacturer to demonstrate product quality and safety, more than half of the studies proposed herein would be unnecessary. However, the current state of regulations places the burden for investigation of botanicals on appropriately trained researchers dedicated to this fascinating field, which requires the convergence of many scientific disciplines and funding by the NIH.

In June 2004, the US National Institutes of Health (NIH) accepted applications for integrated Botanical Research Centers (BRCs). This request for applications (RFA-OD04-002) represented a renewal of a program supported in large part by the NIH National Center for Complementary and Alternative Medicine (NCCAM) and the NIH Office of Dietary Supplements (ODS), now with additional funds provided by the National Institute of Environmental Health Sciences (NIEHS). The program had previously funded six academic centers for the study of botanical agents in a variety of therapeutic areas, including women's health, cardiovascular disease, and diabetes. Since the original establishment of the program and the explosion of dietary supplement use in the U.S., a number of adverse reactions and drug-herb interactions have become increasingly reported in mainstream, peer-reviewed biomedical research journals. Moreover, several high-profile clinical efficacy trials of historically-useful botanical agents resulted in negative outcomes due, in part, to inadequate assurances of the quality, content, and bioavailability of the study agent employed.

The RTI center for research on botanical quality and safety

In order to address emerging safety issues, a team was assembled at Research Triangle Institute (RTI) to propose to NIH a unique approach: a center dedicated to the study of botanical quality and safety rather than study in a specific therapeutic area (Table 1). The RTI BRC would take advantage of a concentration of botanically relevant skills and capabilities that had been built at the Institute over the preceding decades. Moreover, the proposed center would also draw on botanical and clinical researchers that abound in the surrounding area known as Research Triangle Park.

For example, RTI chemists are known for the discovery of the plant-derived antitumor drugs, taxol and camptothecin, isolated from the Pacific yew tree *(Taxus brevifolia)* and the Chinese tree of joy *(Camptotheca acuminata),* respectively (Oberlies and Kroll, 2004). Virtually all of the analytical and preparative techniques used for these agents can be applied easily to the characterization of complex botanical agent preparations. RTI researchers have long provided support to the National Toxicology Program of NIEHS for the provision of reference standards for botanical medicines, assessment of drug metabolizing enzyme interactions (hepatic enzyme induction and inhibition studies), and the bioavailability of botanical active principles in rodent models (Kim et al., 2001; Mathews et al., 2002; Kim et al., 2003; Levine et al., 2004; Oberlies et al., 2004). In-house expertise also includes sensitive analytical technologies for the detection of heavy metal contamination (using inductively-coupled plasma mass spectrometry), prescription drug adulteration, pesticide and herbicide contamination, and microbial and mycotoxin contamination (Akland et al., 2000; Hu et al., 2000; Menetrez and Foarde, 2002; Milstein et al., 2003; Graf et al., 2004; Levine et al., 2004; Menetrez and Foarde, 2004).

Collaborative relationships exist with other hepatic biology experts such as Dr. Edward LeCluyse, then of the University of North Carolina (UNC). Moreover, the group has existing relationships with local and nationally-recognized botanists such as Dr. Peter White, curator of the North Carolina Botanical Garden, and Trish Flaster, M.S., CEO of Botanical Liaisons of Boulder, CO for botanical identification, taxonomy, and the deposition and documentation of voucher specimens.

Clinical experts in the area also agreed to provide support to the group, including UNC's director of their General Clinical Research Center and member of the NIH Drug- Induced Liver Injury Network (DILIN), Dr. Paul Watkins, and Phase I clinical trialists at Duke University Medical Center. With the exception of Ms. Flaster, all expertise required for execution of the Center's research resided within a 10 mile radius.

Although the application was not selected for funding following the competitive evaluation process, NIH reviewers were highly enthusiastic about this interdisciplinary group of laboratories capable of assessing botanical quality and contaminants in noting, "each of these areas is a specialty, so assembling a competent group to perform these disparate tasks would be difficult in another setting."

THE LIVER AS A SITE FOR BOTANICAL-DRUG INTERACTIONS AND TOXICITY

As most botanical supplements are administered orally, the liver and, to a lesser extent, the intestinal mucosa both represent sites at which pharmacokinetic drug-herb interactions can occur. Conjugases, transporters and the monooxygenase cytochrome P450 (P450) are present in the intestine and liver, where they play a major role in presystemic metabolism. P450 is often the preeminent metabolic enzyme in those tissues, and the P450 forms CYP1A2, 2C9, 2C19, 2D6 and 3A4 together account for the metabolism of approximately 90% of all clinically-prescribed drugs (Smith et al., 1998). Accordingly, package inserts included with prescription drugs now report data on the in vitro inhibition of human P450 enzymes as a means of predicting drug interactions mediated by competition for metabolism by the same enzyme. It is anticipated that, in the future, information on interactions with specific intestinal transporters will be included as well because of their marked impact on the bioavailability of the drugs which they transport.

The P450 enzyme family is also of historical relevance in botanical medicine due to its role in the hepatotoxic effects of xenobiotics (Stedman, 2002). Hepatotoxicity is a leading cause of drug-related fatalities, due primarily to the fact that these phase I drug- metabolizing enzymes can bioactivate natural and synthetic

compounds to chemically- reactive metabolites (Villeneuve and Pichette, 2004). Most of the herbs capable of this effect have been removed from the market, although comfrey *(Symphytum officinale)* supplements and teas, known sources of hepatotoxic pyrrolizidine alkaloids, continue to be sold (Kim et al., 2001; Oberlies et al., 2004). Moreover, recent positive clinical trial results with pyrrolizidine alkaloid-free butterbur *(Petasites hybridus)* extract, Petadolex, in migraine prophylaxis (Lipton et al., 2004) suggests that scientists and clinicians should be vigilant in monitoring for hepatotoxic adverse events that may arise from ingestion of lower quality, copycat products that are likely to surface (Kalin, 2003). This issue is of such great importance to stakeholders that the 2003 gathering sponsored by the National Center for Natural Products Research and the Center for Food Safety and Applied Nutrition was entitled, "Hepatotoxicity Assessment for Botanical Dietary Supplements." The reader is referred to the meeting summary (Walker, 2005) authored by Dr. Larry Walker, director of the University of Mississippi National Center for Natural Products Research for a discussion of methods that might be employed to screen botanical supplements for predictable hepatotoxic reactions.

Roughly 50 percent of drug-drug interactions have a pharmacokinetic basis in the liver (and/or intestine) and this has proven to be the case with drug-herb interactions as well. The biology of the cytochrome P450 family was at the heart of several, high-profile drug-herb interactions observed between St. John's wort *(Hypericum perforatum)* extracts and prescription drugs used in life-threatening situations such as HIV/AIDS or in post-organ transplant immunosuppression (Piscitelli et al., 2000; Ruschitzka et al., 2000). We propose that prospective analysis of the acute and chronic effects of St. John's wort extracts on the purified drug-metabolizing enzymes and enzyme activity in cultured human hepatocytes might have prevented the negative press associated with these high profile mishaps.

For example, a major advance in understanding CYP3A4 regulation was the discovery and cloning of the pregnane X receptor transcription factor (PXR) (Kliewer et al., 1998). This orphan nuclear receptor/transcription factor was shown to bind with high affinity several well-known CYP3A4 inducing agents, such as rifampicin, and activate transcription of the CYP3A4 gene via a specific PXR-response element (PRE). Subsequently, Kliewer's group demonstrated that hyperforin from St. John's wort was an even more effective PXR ligand than rifampicin (Moore et al., 2000). Hyperforin was therefore responsible for these CYP3A4-mediated herb-drug interactions that began to surface in 1999 and 2000 whereby chronic St. John's wort use was associated with increased hepatic clearance and subtherapeutic drug levels of the HIV protease inhibitor, indinavir, and the immunosuppressive drug, cyclosporine.

In another cancer-related study, St. John's wort was reported to enhance the clearance of irinotecan (CPT-11; Camptosar®), a semi-synthetic camptothecin used in advanced colorectal cancer (Mathijssen et al., 2002). Patients receiving St. John's wort with irinotecan experienced a 40% decrease in levels of the active metabolite, SN-38, thereby compromising the anticancer effect of the camptothecin. This effect persisted even when St. John's wort was discontinued for three weeks prior to the next course of chemotherapy. Induction of CYP3A4 has been hypothesized as the mechanism by which St. John's wort modulates irinotecan clearance, indicating the potential for numerous interactions between herbal products and cytotoxic chemotherapeutic agents. CYP3A4 is known to metabolize a number of chemotherapy agents, including etoposide (VP-16) and vinca *(Vinca rosea)* alkaloids, and to play a role in the activation of ifosfamide to its alkylating species (Kivisto et al., 1995). Therefore, long-term use of herbal products known to induce the activity of this enzyme, such as St. John's wort, might lead to increased clearance of numerous chemotherapeutic agents and possibly enhance the bioactivation and toxicity of ifosfamide.

Conversely, *acute* exposure to herbal products has been shown to inhibit the activity of CYP3A4 (Budzinski et al., 2000; Obach, 2000; Gaudineau et al., 2004), representing the potential for amplified effects of several cytotoxic agents, such as etoposide or the *Vinca* alkaloids, or prevention of the CYP3A4-dependent activation of ifosfamide. Therefore, the timing and the context of herb use is of particular importance in defming the potential for drug-herb interactions.

ACTUAL COSTS OF ASSESSING DRUG-METABOLIZING ENZYME EFFECTS OF BOTANICALS

A major perceived barrier to prospective analysis of botanical supplement effects on drug-metabolizing enzymes is the cost of these studies. To address this perception, we solicited pricing information from the company of one of our former academic collaborators for these analyses. A complete panel of IC50 and K, inhibition studies for eleven P450 isoforms, monoamine oxidases A and B, aldehyde dehydrogenases, xanthine oxidase, and the UDP-glucuronyltransferase would cost approximately $6,000 per sample. Induction studies for the same enzymes (using enzyme activity or immunoblotting endpoints) in human hepatocytes would cost between $13,000 and $40,000, depending on the degree of complexity of studies required. If any inhibitory or inducing interactions are observed, historical or prospective pharmacokinetic studies could indicate the degree of likelihood that such an interaction might be expected in consumers taking prescription drugs concomitantly. Therefore, a botanical manufacturer could be rule out one major cause of drug-herb interactions for less than $50,000 per supplement. While these studies are not required by the U.S. Food and Drug Administration, the marketing advantage of a product that has been tested for these effects is obvious. Moreover, movement by the industry towards this type of testing would be likely to generate significant goodwill among consumers and health-care providers.

Obviously, the most appropriate setting in which to assess metabolism-based botanical drug-herb interactions is in human subjects. One approach has been the use of model substrates for specific P450 isoforms (Gurley et al., 2002; Gurley et al., 2006) as outlined by the excellent work of Dr. Bill Gurley at the University of Arkansas for Medical Sciences. For this reason, the proposed RTI BRC collaborates with clinical pharmacology colleagues at the University of North Carolina (Dr. Mary Paine) and one of the co-authors (H.S.S.) with others at Duke University Medical Center. These collaborations are essential to extrapolating in vitro studies to the actual setting of the consumer and patient, as well as to establish a framework for determining whether in vitro interactions observed are relevant given the in vivo plasma levels of botanically- derived active principles.

AVOIDING PHARMACODYNAMIC BOTANICAL-DRUG INTERACTIONS

Much of this discussion has focused on drug-herb interactions based on a botanical constituent inducing or inhibiting the metabolism of a drug, thereby reducing or increasing, respectively, the blood levels of a given drug. Depending on the direction of the change, the net effect of the interaction could be increased drug toxicity (especially for low-therapeutic index drugs such as cancer chemotherapeutics) or subtherapeutic blood levels of the drug, such that the patient is essentially undermedicated.

Pharmacodynamic interactions are the result of one agent antagonizing the effect of another at the level of the cellular drug target. For example, our group is involved with two projects at the integrative oncology program for children's cancer at Columbia University led by Kara Kelly, M.D. and Elena Ladas, M.S., R.D. The projects include a clinical trial of a milk thistle *(Silybum marianum)* extract in prevention of liver toxicity of methotrexate chemotherapy in pediatric cancer patients, while the second is a clinical trial of glutamine supplementation to prevent peripheral neuropathy of vincristine chemotherapy. In both cases, it is essential to assure that side effect mitigation by supplement does not compromise desired anticancer activity of drug regimen. Using in vitro cancer cell survival experiments, we have confirmed that glutamine supplementation to concentrations expected in patients is without effect on the antileukemic activity of vincristine, L-asparaginase, or methotrexate chemotherapy. Similarly, milk thistle extracts have no effect on L-asparaginase or methotrexate efficacy.

Hence, in no case were the supplements expected to interfere with the desired therapeutic effect of the antileukemic drug regimen. In fact, a surprising and useful observation arose from these studies, in that milk thistle extracts may synergize with vincristine in killing leukemia cells (Nakanishi et al., manuscript in preparation). Therefore, studying potentially negative drug-herb interactions also has the potential for revealing novel combinations that may enhance drug efficacy. Obviously, our group is currently pursuing funding sources to further investigate this potential synergy of milk thistle and vincristine in an appropriate model of human childhood leukemias.

THE CASE OF CASE REPORTS

Most botanical adverse reactions and drug-herb interactions first become apparent through publication of case reports. In particular, poison control centers have become important sentinels of adverse reactions and interactions (Dennehy et al., 2005). However, case reports are anecdotes and do not establish causality between a given botanical agent and the adverse reaction reported (Clouatre, 2004). The value of case reports is as hypothesis-generators.

For example, we reported in 1998 on a case where use of an undefined St. John's wort extract by an asthmatic patient resulted in dramatically increased clearance of the oral bronchodilator, theophylline (Nebel et al., 1999). This association was supported by cell culture findings that hypericin in St. John's wort was capable of inducing activity via a major gene transcription element for CYP1A2. While work was ongoing to determine the causality of the interaction, we published a review article in the *Journal of Herbal Pharmacotherapy* (Kroll, 2001) that bears quoting to emphasize the need for objectivity in evaluating the safety of herbal products:

A less obvious area of negative bias is the publication of case reports. While valuable as flags for potential adverse effects and starting points for further studies, case reports on side effects due allegedly to herbal products have been used to indict the entire herbal medicine industry. Interestingly, we in conventional medicine urge our patients not to rely on anecdotal reports of efficacy of herbal products due to lack of blinding, placebo effects, and lack of consideration of the natural course of the disease. However, case reports (even the one published by this author on the theophylline-St. John's wort interaction) should also be viewed as anecdotal until substantiated further. (Kroll, 2001)

Since our case report, two clinical studies have been published to follow-up on this finding. Gurley et al. (2002) used paraxanthine/caffeine phenotypic ratios to assess CYP1A2 activity in normal human volunteers before and after being given a St. John's wort supplement standardized to 0.3% hypericin, three times daily, for 28 days. The overall trend revealed a statistically-significant increase in CYP1A2 activity. However, six of the twelve individuals experienced no change in activity while the other six each experienced increases greater than the mean increase. A second study performed in twelve healthy males in Japan measured theophylline and its metabolites in plasma and urine after 14 days of St. John's wort extract at the same dose (Morimoto et al., 2004). No significant changes were observed in any parameter. The conclusion that can be drawn is that St. John's wort may increase CYP1A2 activity in a subset of patients, but this increase is likely to be less dramatic than with the most intensively studied induction of CYP3A4. A factor that may account for the interindividual differences in the study of Gurley et al. and in our case report is that smoking produces polyaromatic hydrocarbons that might act in concert with hypericin in St. John's wort to increase CYP1A2 activity. Indeed, the patient in the case report was a cigarette smoker.

What Should Happen Ideally After Botanical Adverse Reaction/Drug Interaction Case Reports Appear?

As the case above illustrates, a rush to judgment is inappropriate until further studies are executed. The case report fueled discussions on the likelihood of causality between St. John's wort constituents and CYP1A2 induction leading up to the generation of testable hypotheses in human volunteers in two countries. Ideally, test compound and representative extracts should be evaluated in progressively more relevant systems, from hepatocytes to whole animals to human studies. Potentially offending compounds from the botanical in question should also be tested for their bioavailability in progressively more relevant systems (Caco-2 permeability > rodent models > human studies) and mechanistic studies should follow (Clouatre, 2004; Zou et al., 2004). Wherever possible in vivo studies should proceed at physiologically relevant dosing, recognizing allometric scaling issues between rodents and humans (where 1 mg/kg in humans = 7 mg/kg in rats = 12 mg/kg mice).

Most important is that botanical studies in human volunteers be expanded to include measurements of plasma concentrations of the purported pharmacologically- or toxicologically-relevant compound to determine whether the concentrations of compounds that elicit cell culture effects can even be achieved in an intact biological system or human volunteer. This point is important in interpreting safety issues of a botanical compound, since any number of effects can be observed in vitro that bear no relevance to plasma or tissue concentrations achieved in vivo. We have previously urged this point in efficacy trials, so that reports of negative outcomes

could be correlated with whether botanical constituents achieved expected effective concentrations in volunteers. Similarly, botanical manufacturers may wish to have these data to defend against criticism of their products (i.e., a negative effect in vitro would be of no relevance if plasma levels of the offending compound are 1000-fold lower when the botanical is taken at a recommended dose).

THE ROLE OF MEDIA TRAINING IN THE CAREER DEVELOPMENT OF BOTANICAL RESEARCHERS

As a result of the intense public interest in botanical medicinal products, positive and negative research results are often the topic of mainstream press coverage. Barry Glassner's book, *The Culture of Fear: Why Americans Are Afraid of the Wrong Things* (Glassner, 1999), illustrates the interest of the press in seizing upon stories that have a sensationalistic aspect independent of their actual relative risk to human health. Therefore, the experience of the authors is that a botanical researcher is more likely to be contacted by the press than a research in another area whose work may have less immediate impact on human health. Therefore, it is important to provide some level of media training at all levels of career development in botanical research, not only to protect one's research message but also as an educational opportunity for the general public.

We are fortunate to reside in a geographical area with a density of accomplished media figures dedicated to education of the public and have tapped these experts to provide media workshops to our trainees. Joe and Terry Graedon of National Public Radio's *The People's Pharmacy* have agreed to provide a workshop on radio interviewing. A television news workshop would be given by Tom Linden, MD, director of the University of North Carolina graduate program in medical journalism, and Helen Chickering, a reporter and producer for the national NBC News Channel portal. Interactions with the print news media would be covered by Tracey Koepke of the Duke University Medical Center News Office, along with others. Finally, like other institutions, our own office of public relations and corporate communications offers individualized press training.

Appropriate training of scientists in interacting with the press can help to refine the message delivered to the public and increase the probability that an accurate representation of one's research results is disseminated.

CONCLUSIONS

A plan has been proposed to create an independent, non-profit, national center for research on botanical quality and safety to prevent the cases of misfortune that have plagued the integration of botanical medicine in the US healthcare system. Dietary supplement manufacturers enjoy a modest level of scrutiny by the US Food and Drug Administration, but a case has been made that voluntary adoption of basic preclinical and clinical evaluation of herbal products for quality and safety could have prevented the major adverse reactions, drug-herb interactions, and cases of product adulteration and contamination that have brought negative publicity to the industry. Voluntary adoption of suggested guidelines would also serve many intangible goals by increasing goodwill and acceptance among the American medical community and promoting trust among the consuming public.

ACKNOWLEDGMENTS

Research leading to the submission of the RTI BRC proposal was supported by R21 CA100721 from the Office of Cancer Complementary and Alternative Medicine of the US National Cancer Institute (to D.J.K., N.H.O., and J.M.M.). H.S.S. is an RTI visiting scientist supported by a K08 mentored physician-scientist career development award (K08 AT001315) from the National Center for Complementary and Alternative Medicine. D.J.K. also expresses thanks to Dr. Bill Gurley (UAMS) and Dr. Christopher Borgert (Applied Pharmacology & Toxicology, Gainesville, FL) for thoughtful discussions during the Oxford Conference.

The authors wish to express their gratitude to the entire research team who participated in the submission of our botanical research center proposal: Drs. Jim Stephenson, Joel Sevinsky, Edo Pellizzari, Keith Levine, Jim Raymer, Charles Sparacino, Amy Etheridge, Karin Foarde, Jon Black, and Bernadette Marriott from RTI, Dr. Maria Rapoza from the North Carolina Center for Biotechnology, Drs. Peter White, Mary Paine, and Paul Watkins from UNC, and Trish Flaster from Botanical Liaisons (Boulder, CO). The authors are also grateful to

Dr. Ed LeCluyse and Shiloh Barfield from CellzDirect (Pittsboro, NC) for providing expertise and pricing information on liver enzyme and cell procurement services for presentation at the conference and use in this manuscript.

Literature Cited

Akland, G.G., Pellizzari, E.D., Hu, Y., Roberds, M., Rohrer, C.A., Leckie, J.O. and Berry, M.R. 2000. Factors influencing total dietary exposures of young children. J. Expo. Anal. Environ. Epidemiol. 10:710-722.

Barnes, P.M.. Powell-Griner, E., McFann, K., and Nahin, R.L. 2004. Complementary and Alternative Medicine Use Among Adults: United States, 2002. National Center for Health Statistics. CDC Advance Data Report #343.

Bent, S., Kane, C., Shinohara, K., Neuhaus, J., Hudes, E.S., Goldberg, H. and Avins, A.L. 2006. Saw palmetto for benign prostatic hyperplasia. N. Engl. J. Med. 354:557-566.

Budzinski, J.W., Foster, B.C., Vandenhoek, S. and Amason, J.T. 2000. An in vitro evaluation of human cytochrome p450 3a4 inhibition by selected commercial herbal extracts and tinctures. Phytomedicine 7:273-282.

Clouatre, D.L. 2004. Kava kava: Examining new reports of toxicity. Toxicol. Lett. 150:85-96.

Dennehy, C.E., Tsourounis, C. and Horn, A.J. 2005. Dietary supplement-related adverse events reported to the California poison control system. Am. J. Health. Syst. Pharm. 62:1476-1482.

Ernst, E. 2002. Adulteration of Chinese herbal medicines with synthetic drugs: A systematic review. J. Intern. Med. 252:107-113.

Gaudineau, C., Beckerman, R., Welboum, S. and Auclair, K. 2004. Inhibition of human p450 enzymes by multiple constituents of the Ginkgo biloba extract. Biochem. Biophys. Res. Commun. 318:1072-1078.

Glassner, Barry. The culture of fear: why Americans are afraid of the wrong things. NY: Basic books.

Graf, T.N., Levine, K.E., Perlmutter, J., Andrews, M.E., Davis, J.M. and Oberlies, N.H. (2004). Analyzing the yield of isoquinoline alkaloids in cultivated and wild crafted bloodroot (Sanguinaria canadensis) grown in North Carolina. International Congress on Natural Products Research, Phoenix, AZ.

Gurley, B., Hubbard, M.A., Williams, D.K., Thaden, J., Tong, Y., Gentry, W.B., Breen, P., Carrier, D.J. and Cheboyina, S. 2006. Assessing the clinical significance of botanical supplementation on human cytochrome p450 3a activity: Comparison of a milk thistle and black cohosh product to rifampin and clarithromycin. J. Clin. Pharmacol. 46:201-213.

Gurley, B.J., Gardner, S.F., Hubbard, M.A., Williams, D.K., Gentry, W.B., Cui, Y. and Ang, C.Y. 2002. Cytochrome p450 phenotypic ratios for predicting herb-drug interactions in humans. Clin. Pharmacol. Ther. 72:276-287.

Hu, Y.A., Barr, D.B., Akland, G., Melnyk, L., Needham, L., Pellizzari, E.D., Raymer, J.H. and Roberds, J.M. 2000. Collecting urine samples from young children using cotton gauze for pesticide studies. J. Expo. Anal. Environ. Epidemiol. 10:703-709.

Kalin, P. 2003. [the common butterbur (Petasites hybridus)--portrait of a medicinal herb]. Forsch. Komplementarmed. Klass. Naturheilkd. 10 Suppl. 1:41-44.

Kim, N.-C., Graf, T.N., Sparacino, C.M., Wani, M.C. and Wall, M.E. 2003. Complete isolation and characterization of silybins and isosilybins from milk thistle (Silybum marianum). Org. Biomol. Chem. 1:1684-1689.

Kim, N.-C., Oberlies, N.H., Brine, D.R., Handy, R.W., Wani, M.C. and Wall, M.E. 2001. Isolation of symlandine from the roots of common comfrey (Symphytum officinale) using countercurrent chromatography. J. Nat. Prod. 64:251-253.

Kivisto, K.T., Kroemer, H.K. and Eichelbaum, M. 1995. The role of human cytochrome p450 enzymes in the metabolism of anticancer agents: Implications for drug interactions. Br. J. Clin. Pharmacol. 40:523-530.

Kliewer, S.A., Moore, J.T., Wade, L., Staudinger, J.L., Watson, M.A., Jones, S.A., McKee, D.D., Oliver, B.B., Willson, T.M., Zetterstrom, R.H., Perlmann, T. and Lehmann, J.M. 1998. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. Cell 92:73-82.

Kroll, D.J. 1997. Addressing the barriers to acceptance of herbs as medicines. Keynote address. Natural Products Expo East, Sept 17-21, Baltimore, MD, U.S.A.

Kroll, D.J. 2001. Concerns and needs for research in herbal supplement pharmacotherapy and safety. J. Herb. Pharmacotherapy 1:3-21.
Kroll, D.J. and Oberlies, N.H. 2003. The impact of newly proposed dietary supplement manufacturing guidelines on patient safety and clinical trials outcomes. Focus on Alternative and Complementary Therapies 8:302-306.
Lau, A.J., Holmes, M.J., Woo, S.O. and Koh, H.L. 2003. Analysis of adulterants in a traditional herbal medicinal product using liquid chromatography-mass spectrometry- mass spectrometry. J. Pharm. Biomed. Anal. 31:401-406.
LeCluyse, E., Madan, A., Hamilton, G., Carroll, K., DeHaan, R. and Parkinson, A. 2000. Expression and regulation of cytochrome p450 enzymes in primary cultures of human hepatocytes. J. Biochem. Mol. Toxicol. 14:177-188.
Levine, K.E., Fernando, R.A., Milstein, L.S., Clark, T., Essader, A., Ross, G.T. and Pellizzari, E.D. 2002. Trace element content of commercially available dietary supplements as determined by inductively coupled plasma optical emission spectrometry and inductively coupled plasma mass spectrometry. J. Am. Nutraceutical Assoc. 5:5-13.
Levine, K.E., Ross, G.T., Fernando, R.A., Blake, J.C., Sparacino, C.M. and Pellizzari, E.D. 2004. Trace element content of senna study material and senna-based dietary supplements as determined by inductively coupled plasma optical emission spectrometry and inductively coupled plasma mass spectrometry. Comm. Soil Sci. Plant Anal. 35:835-851.
Lipton, R.B., Gobel, H., Einhaupl, K.M., Wilks, K. and Mauskop, A. 2004. Petasites hybridus root (butterbur) is an effective preventive treatment for migraine. Neurology 63:2240-2244.
Mathews, J.M., Etheridge, A.S. and Black, S.R. 2002. Inhibition of human cytochrome p450 activities by kava extract and kavalactones. Drug Metab. Dispos. 30:1153-1157.
Mathijssen, R.H., Verweij, J., de Bruijn, P., Loos, W.J. and Sparreboom, A. 2002. Effects of St. John's wort on irinotecan metabolism. J. Nat. Cancer Inst. 94:1247-1249.
Menetrez, M.Y. and Foarde, K.K. 2002. Microbial volatile organic compound emission rates and exposure model. Indoor and Built Environment 11:208-213.
Menetrez, M.Y. and Foarde, K. 2004. Research and development of prevention and control measures for mold contamination. Indoor and Built Environment 13:109-114.
Milstein, L.S., Essader, A., Pellizzari, E.D., Fernando, R.A., Raymer, J.H., Levine, K.E. and Akinbo, O. 2003. Development and application of a robust speciation method for determination of six arsenic compounds present in human urine. Environ. Health Perspect. 111:293-296.
Moore, L.B., Goodwin, B., Jones, S.A., Wisely, G.B., Serabjit-Singh, C.J., Willson, T.M., Collins, J.L. and Kliewer, S.A. 2000. St. John's wort induces hepatic drug metabolism through activation of pregnane x receptor. Proc. Nat. Acad. Sc. 97:7500-7502.
Morimoto, T., Kotegawa, T., Tsutsiimi, K., Ohtani, Y., Imai, H. and Nakano, S. 2004. Effect of St. John's wort on the pharmacokinetics of theophylline in healthy volunteers. J Clin Pharmacol 44:95-101.
Nebel, A., Schneider, B.J., Baker, R.K. and Kroll, D.J. 1999. Potential metabolic interaction between St. John's wort and theophylline. Annals of Pharmacotherapy 33:502.
Obach, R.S. 2000. Inhibition of human cytochrome p450 enzymes by constituents of St. John's wort, an herbal preparation used in the treatment of depression. J. Pharmacol. Exp. Ther. 294:88-95.
Oberlies, N.H., Kim, N.-C., Brine, D.R., Collins, B.J., Handy, R.W., Sparacino, C.M., Wani, M.C. and Wall, M.E. 2004. Analysis of herbal teas made from the leaves of comfrey (Symphytum officinale): Reduction of n-oxides results in order of magnitude increases in the measureable concentration of pyrrolizidine alkaloids. Pub. Health Nutr. 7:919-924.
Oberlies, N.H. and Kroll, D.J. 2004. Camptothecin and taxol: Historic achievements in natural products research. J. Nat. Prod. 67:129-135.
Piscitelli, S.C., Burstein, A.H., Chaitt, D., Alfaro, R.M. and Falloon, J. 2000. Indinavir concentrations and St John's wort. Lancet 355:547-548.
Pittler, M.H. and Ernst, E. 2003. Systematic review: Hepatotoxic events associated with herbal medicinal products. Aliment. Pharmacol. Ther. 18:451-471.

Ruschitzka, F., Meier, P.J., Turina, M., Luscher, T.F. and Noll, G. 2000. Acute heart transplant rejection due to Saint John's wort. Lancet 355:548-549.
Saper, R.B., Kales, S.N., Paquin, J., Burns, M.J., Eisenberg, D.M., Davis, R.B. and Phillips, R.S. 2004. Heavy metal content of ayurvedic herbal medicine products. JAMA 292:2868-2873.
Smith, D.A., Abel, S.M., Hyland, R. and Jones, B.C. 1998. Human cytochrome p450s: Selectivity and measurement in vivo. Xenobiotica 28:1095-1128.
Stedman, C. 2002. Herbal hepatotoxicity. Semin. Liver Dis. 22:195-206.
Turner, R.B., Bauer, R., Woelkart, K., Hulsey, T.C. and Gangemi, J.D. 2005. An evaluation of Echinacea angustifolia in experimental rhinovirus infections. N. Engl. J. Med. 353:341-348.
Villeneuve, J.P. and Pichette, V. 2004. Cytochrome p450 and liver diseases. Curr. Drug Metab. 5:273-282.
Walker, L.A. 2005. Hepatotoxicity assessment for botanical dietary supplements. HerbalGram 65:64-66.
Wolsko, P.M., Solondz, D.K., Phillips, R.S., Schachter, S.C. and Eisenberg, D.M. 2005. Lack of herbal supplement characterization in published randomized controlled trials. Am. J. Med. 118:1087-1093.
Zou, L., Harkey, M.R., Henderson, G.L. and Dike, L.E. 2004. Kava does not display metabolic toxicity in a homogeneous cellular assay. Planta. Med. 70:289-292.

Table 1. Components of a Botanical Research Center Designed to Assure Quality and Safety.

Natural Products Characterization Core
- Botanical verification
- Quantification of biologically-active constituents
- Qualify study extracts and provide pure compounds
- Heavy metals
- Pesticides and herbicide residues
- Microbial contamination and mycotoxins
- Adulteration with common pharmaceuticals

Drug Metabolism and Pharmacokinetics
- Assessment of potential hepatotoxicity
- Hepatic-based botanical drug interactions
- Bioavailability of key products
- Potential to move to phase I preclinical trials

Pharmacology and Proteomics
- Assure lack of pharmacodynamic interaction with drugs likely to be used in a specific therapeutic setting
- Investigate proteomic profile for drug or herb hepatotoxicity and action of hepatoprotective botanicals
- Not intended to address other aspects of in vivo efficacy (focus of other botanical research centers)

Career Development and Media Training
- Pilot grant program to encourage well-trained junior faculty members in applying technology to important questions in botanical medicine
- Training in appropriate application of case reports to hypothesis-generated research questions
- Presenting a clear message to the public outlets on the proper interpretation and extrapolation of basic and clinical research results