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Research Article

Assessment of Dong Quai Hepatic Metabolism and Potential Interactions when Combined with Chemotherapy

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Abstract

Background: Dong Quai is a common herbal supplement classified as a "phytoestrogen" used for the improvement of female reproductive function. In the oncology setting, women often seek natural approaches for managing symptoms associated with decreased hormone levels either from surgery or chemotherapy-induced. Clinically, the concern is the safety of phytoestrogens in combination with chemotherapy. The objective of this study was to characterize the hepatic metabolism of Dong Quai to define the potential for drug interactions with selected chemotherapy agents and its impact on alterations in the cytotoxicity in panel of human cancer cell lines.

Methods: In vitro high through-put cytochrome P450 (CYP450) inhibition assay was performed for CYP450 2C9, 2C8, 2D6 and 3A4 isoenzymesto evaluate phase I metabolism of Dong Quai alcohol-free extract. An ex vivo hepatic induction assay with human hepatocytes was used to determine whether Dong Quai is an inducer of CYP450 isoenzymes. The potential cytotoxic effects of Dong Quai alone and its effect when combined with selected chemotherapies were evaluated by a growth inhibition assay in a panel of eight human cancer cell lines.

Results: No inhibition of CYP450 was observed in presence of Dong Quai. At an estimated clinical relevant concentration of 0.86 mg/mL, Dong Quai demonstrated Quai induced CYP3A4, 2C9, 2C8 and 2D6. Dong Quaidid not demonstrated cytotoxicity by itself in the panels of eight human cancer cell lines with 50% growth inhibition was not achieved. The 25% growth inhibition was achieved at concentrations ranging from 0.39 mg/mL to 4.48 mg/mL. Combination growth inhibition assays showed decreased cytotoxic activity of chemotherapy agents.

Conclusion: This data suggests that Dong Quai is an inducer of the CYP450 pathways and also decreased cytotoxic activity of selected chemotherapy. Until confirmatory in vivo information available Dong Quai should be used with caution with chemotherapy.

Keywords: Dong Quai; Cytochrome P450; Phytoestrogen; Metabolism; Cancer; Chemotherapy; Drug interactions

Introduction

Herbal and nutritional supplements have become very popular form of the Complementary Alternative Medicine (CAM) used to enhance health, disease prevention, and as "natural" alternatives to treat a number of the symptoms including hot flashes, decrease libido, vaginal dryness or insomnia associated with conditions with hormone imbalances such as menopause. Historically, synthetic hormones products have been prescribed together as Hormone Replacement Therapy (HRT) to manage the symptoms associated with menopause. Since its inception, the use of HRT in postmenopausal women in western countries has consistently increased over time until the concerning reports from five large randomized trials that evaluated HRT therapy in over 40,000 women that led to developments of clinical controversy on its role in management of menopause [1-6]. The major conclusions from all five of these trials was a significant and potentially fatal risk of using HRT for more than five years, including an increased incidence of breast cancer, stroke, and pulmonary embolism [1-6]. The Women's Health Initiative study was the largest amongst these studies, randomizing 16,608 women to HRT versus placebo that an interim analysis found there was an increased risk of breast and endometrial cancer, as well as significant increase risk thromboembolism associated with long-term use of HRT and lead to early closure of this study [3]. The symptoms of menopause are not life-threatening but can have quite the impact on quality of life and daily routines. Hence many women have sought natural alternatives to HRT specifically the herbal supplements classified as phytoestrogens.

Phytoestrogen is a term used to describe a group of natural products with a historic use for conditions that are now treated by exogenous estrogens. In general, phytoestrogens are biologically active compounds of plant origin with proposed estrogenic activity, evident by reported data to control menopausal symptoms such as hot flushes and mood swings. There are over eighteen phytoestrogen products based their plant family names in current consumers market. This study focused on one of the most commonly used phytoestrogen, Dong Quai, which if found in many combination herbal supplement products designed for women to use to manage hormone-related symptoms. Dong Quai also known as *Angelica sinensis* is a perennial herb native to China, Japan, and Korea. For thousands of years in traditional Far Eastern medicine, Dong Quai has been widely used for correcting menstrual disorders and other hormone-related symptoms and is often combined with other herbs [7,8].

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Current pre-clinical and clinical studies on the safety of phytoestrogens like Dong Quai are very conflicting. While in some pre-clinical *in vitro* studies phytoestrogens have been reported to stimulate cancer cell growth, this has often not been confirmed with *in vivo* or clinical studies. While some preclinical studies *in vitro* studies showed that Dong Quai significantly stimulated the proliferation of MCF-7 cells, clinically use of Dong Quai has been deemed safe but not always effective [9-11]. Part of the contributing factors for the conflicting data is the inconsistency in dosing, specifically using doses in preclinical studies that is unachievable, magnitudes of concentrations that could be achieved in humans. To date though, there is very limited if any pharmacokinetic data available for any of the phytoestrogens including Dong Quai. Thus, in these studies an "estimated clinically relevant dose" was used. This concentration assumes 100% bioavailability in an average 70 g adult with 7 L volume of distribution. It is not exact, but was deemed appropriate concentration to use to evaluate the metabolism of Dong Quaiand to evaluate the drug interaction between Dong Quai and several selected chemotherapy agents including the potential impact oncytotoxicity of Dong Quai with or without chemotherapy in a panel of human cancer cell lines. The long term goal of this study is to contribute to the investigation to identify a safe and effective phytoestrogen to manage hormone-related symptoms in women with cancer receiving active chemotherapy treatment.

Materials and Methods

High throughput cyp450 inhibition assays

The protocol was adapted from a validated method from BD Gentest (Woburn, MA), the High Throughput Method for Measuring CYP450 Inhibition (version 4.2, 2000) [12,13]. Dong Quai was diluted in deionized water. Acetonitrile was used as solvent to dissolve the positive controls (ketoconazole, quercetin, sulfaphenazole, and quinidine) and substrates [dibenzylfluorescin (DBF) and 3-[2-N,N-diethyl-N-methylammonium) ethyl]-7-methoxy-4-methylcoumarin iodide (AMMC)] (Table 1). Working solutions were made by dilution in 0.5 M potassium phosphate buffer, pH 7.4. Assays were conducted in 96-well black bottom microtiter plates. The final cofactor concentrations were 1.3 mM $NADP^+$, 3.3 mM glucose-6-phosphate, and 0.4 U/mL glucose-6-phosphate dehydrogenase, and 3.3 mM magnesium ion. Final incubation volume for each well was 0.2 mL. After the addition of inhibitor positive control vehicles or Dong Quai at a maximum concentration of 0.86 mg/mL was added (Dong Quai ranged from 0.86 down to 0.00039 mg/mL) (Table 1). The 0.86 mg/mL concentration was selected as an estimate of the clinical relevant concentration based on the current maximum recommended dosage of 6 gram daily as instructed by the manufacturer, assuming 100% bioavailability, and 7 L as the estimated total blood volume of an average adult. Respective enzyme and substrate mixtures (DBF at 100 µM or AMMC at 500 µM) were added to the reaction mixture as appropriate. In each reaction well there were 5, 4, 2 or 1.5 pmol present of CYP450 3A4, 2C8, 2C9, and 2D6, respectively. After one hour incubation at 37°C, reactions were stopped with 75 µL 2 mM sodium hydroxide or 80:20 acetonitrile: tris base solution for CYP450 2D6 only. The extent of CYP450 inhibition was evaluated by comparing the metabolism of varied concentrations in the presence and absence of the known inhibitor. The amount of product metabolized for the control comparison reactions was measured with FL600 Dual-Band plate reader from BioTek Instruments, Inc. (Winooski, VT) using fluorescence emission detection at 528 nm (excitation 485 nm) of fluorescein (metabolite product of DBF metabolism by CYP450) or at 460 nm (excitation 360) of 3-[2-(N, N-diethyl-N-methyl ammonium) ethyl]-7-hydroxy-4-methylcoumarin (AMHC) (metabolite of AMMC).

CYP450 inhibitor	Fluorometric substrate	Excitation/ Emission wavelength (nm)	CYP450 pathway inhibited	Inhibitor concentration	Reaction time
Ketoconazole	DBF	485/528	3A4	0 to 7.5 µM	60 minutes
Quercetin	DBF	485/528	2C8	0 to 15 µM	60 minutes
Sulfaphenazole	DBF	485/528	2C9	0 to 15 µM	60 minutes
Quinidine	AMMC	360/460	2D6	0 to 0.75 µM	60 minutes

Four major CYP450 isoenzymes were selected to determine if Dong Quai is an inhibitor of this system. Ketoconazole, quercetin, sulfaphenazole, and quinidine are known inhibitors of the 4 isoenzymes, here used as positive controls at 100µM. CYP450 isoenzymes transform substrates (DBF and AMMC) to fluorescein metabolites, generating strong signals monitored at responsive excitation/emission wavelengths by FL600 Dual-Band plate reader. The total incubation time was 60 minutes.

Table 1: Positive control inhibitors used in the *in vitro* CYP450 metabolism inhibition study.

CYP450 substrate	CYP450 pathway	Substrate concentration	Wavelength (nm)
Diclofenac	2C8/2C9	100 µM	280
Dextromethorphan	2D6	100 µM	280
Docetaxel	3A4	100 µM	230
Dong Quai	*Test*	0.86 mg/mL	ex360/em460

In CYP450 induction assay, Diclofenac, Dextromethorphan and Docetaxel were used as substrates of different isoenzymes. The substrates were added after 3 day induction of hepatocytes. Working concentrations and wavelength of UV absorption of the substrates are listed. To determine if Dong Quai is a substrate of CYP450 system, it was added in a separate plate in parallel with other substrates. Excitation and emission wavelength of Dong Quai was 360 and 460 nm respectively.

Table 2: CYP450 substrates used in the ex vivo hepatocyte induction assay.

Hepatic metabolism induction assay

Cryopreserved human hepatocytes were purchased from BD Biosciences (Gentest™): Discovery Labware (Woburn, MA). Hepatocytes were allowed to seed for 2 to 4 hours with Hepatozyme SFM media (Gibco™ Invitrogen Corporation, Carlsbad, CA), containing 10% fetal bovine serum (Gemini Bio-Products, West Sacramento, CA) and 250 µM ascorbic acid (Sigma-Aldrich, St. Louis, MO). After adherence was achieved the hepatocytes were maintained in un-supplemented Hepatozyme media and were incubated for 72 hours at 37°C (5% CO_2) to allow recovery before use. An *ex vivo* human metabolism model was used to determine if Dong Quai is able to induce isoenzyme CYP450 3A4, 2C8/2C9, or 2D6. Known substrates for each isoenzymes were used including docetaxel (CYP450 3A4), diclofenac (CYP450 2C8/2C9), and dextromethorphan (CYP450 2D6) (Table 2). The assay was performed in quadruplicate comparing Dong Quai 0.86 mg/mL with a control inducer, rifampicin 25 µM (Sigma-Aldrich, St. Louis, MO) Hepatocytes were induced for 72 hours with either rifampicin or Dong Quai which was supplemented in fresh media every 24 hours. The substrate control treated hepatocytes were supported with only media changes every 24 hours. After 72 hours, rifampicin and Dong Quai were removed and the appropriate concentration of substrate (specific to the CYP450 isoenzyme of interest) was added or Dong Quai as test substrate was added (Table 2). Samples from time 0, 2, 4, 6, and 24 hours were obtained in UV-Vis 96-well plates for CYP450 2C8/2C9, 3A4 and CYP450 2D6 and absorbance were read at 280 nm (Diclofenac and Dextromethorphan) or 230 nm (Docetaxel). To identify if Dong Quai metabolism was induced by rifampicin or Dong Quai extract itself, black 96-well plate was used and detected at 360 nm (excitation) ~460 nm(emission) for fluorescent signal alteration.

Chemicals and drugs

Dong Quai alcohol-free fluid extract was available in 1000 mg/mL solution and was purchased from Nature's Answer (Hauppauge, NY). Quercetin, Quinidine, Ketoconazole, Sulfaphenazole, Dibenzyl Fluorescin (DBF), AMMC, potassium phosphate monobasic, potassium phosphate dibasic, $NADP^+$, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, magnesium chloride hexahydrate, sodium citrate, tribasic, and acetonitrile were all obtained from Sigma-Aldrich (St. Louis, MO). Tris base and 96-well micro titer plates (black/clear bottom) were purchased from Fisher Scientific (Pittsburgh, PA). CYP450 3A4, 2C8, 2C9, and 2D6 isoenzymes microsomes were purchased from BD Biosciences (Gentest™, Bedford, MA) and they were stored at -80°C until analysis and used according the supplier's bulletin. Dimethyl Sulphoxide (DMSO) and 3-(4,5-cimethylthiazol-2-yl)-2,5-diphenyl Tetrazolium bromide (MTT) were purchased from Sigma-Aldrich Co. (St. Louis, MO). Commercial grade chemotherapy agents were used including: carboplatin (SICOR Pharmaceuticals, Irvine, CA), doxorubicin HCl (lyophilized, Ben Venue Laboratories, Inc., Bedford, OH), gemcitabine (GEMZAR®, Eli Lilly and Company, Indianapolis, IN), paclitaxel (TAXOL®, Bristol-Myers Squibb Company, Princeton, NJ) and topotecan (HYCAMTIN®, GlaxoSmithKline, Research Triangle Park, NC)

Cell culture

A panel of human carcinoma cell lines was selected based on Estrogen Receptor (ER) expression including: two ovarian cancer cell lines -SKOV3, TOV-112D; two endometrial cancer cell lines-HEC-1B, Ishikawa; two cervical cancer cell lines-HeLa, SiHa, and one breast cancer cell line, MCF-7, then the NCI-H23, a human non-small cell lung cancer, was included as a ER-negative control. All cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD) and maintained for less than 10 passages. All cell lines were grown in 75 cm^2 culture flasks in 5% CO_2 in air at 37°C to 90% confluence. The 75 cm^2 culture flasks, serological pipettes, 96-well plates and other cell culture supplies were purchased from Fisher Scientific (Pittsburgh, PA).

The cell lines: HEC-1-B (endometrial adenocarcinoma), HeLa (cervical squamous carcinoma), and SiHa (cervical squamous carcinoma) were propagated in a medium consisting of minimum essential medium (MEM) (Eagle's) (Mediatech Inc., Manassas, VA) supplemented with non-essential amino acids (NEAA) and Earle's BSS adjusted to contain 1.0 mM sodium pyruvate, 1.5 g/L sodium bicarbonate, 2 mM L-glutamine, 10% FBS, and 0.4% antibiotic solution (Antimycotic solution-10,000 I.U/mL Penicillin, 10,000 µg/mL Streptomycin, 25 µg/mL Amphotericin B, Mediatech Inc., Manassas, VA 20109). The ovarian adenocarcinoma (SKOV-3) was propagated with media consisting of McCoy's 5a media with 1.5 mM L-glutamine, 10% FBS, and 0.4% mL antibiotics. The mixed adenocarcinoma-endometroid cell line TOV-112D was propagated in a 1:1 mixture of MCDB 105 and medium 199 with 15% FBS and 0.4% mL antibiotics. The endometrial cell line Ishikawa was propagated with 1:1 mixture of DMEM: F12 (low glucose) with 10% FBS. The MCF-7 cell line was propagated with EMEM with 2 mM L-glutamine and Earle's BSS adjusted to contain 1.5 g/L sodium bicarbonate, 0.1 mM NEAA, 1.0 mM sodium pyruvate, 0.01 mg/µL bovine insulin, 10% FBS, and 1% antibiotics The NCI-H23 cell line was propagated with RPMI 1640 with 2 mM L-glutamine, 10 mM Hepes, 1 mM sodium pyruvate, 4.5 g/L glucose, 1.5 g/L sodium bicarbonate, 10% FBS, and 1% antibiotics.

Growth inhibition assays

Growth inhibition assays were performed to determine the cytotoxic activity of Dong Quai alone and in combination with selected chemotherapy agents. Cells were plated to achieve 2,500-5,000 cells per well and incubated at 37°C for 24 hours before treatment. Cells were treated with concentrations ranging from 0.017-8.6 mg/mL of Dong Quai alone using respective cell media as the diluents for dilutions. Control wells had no drug and media alone and blank wells had no cells, drug, or media. A 3 mg/mL stock solution of MTT was prepared using Phosphate-Buffered Saline (PBS). After 72 hours incubation period, 25 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was added to obtain a final concentration of 0.3 mg/mL per well, and cells were further incubated for 2 hours at 37°C. The plates were centrifuged and the supernatant was removed. 50 µL of DMSO was added to all wells and absorbency was measured at 562 nm. The inhibitory concentration to achieve 10% cell death (IC_{10}), IC_{25}, and IC_{50} of Dong Quai for each cell line was calculated. All experiments were done in triplicate for each cell line and drug combination.

To evaluate the potential of Dong Quai to impact the cytotoxic activity, growth inhibition assays were completed with selected commonly used chemotherapy agents including: carboplatin, doxorubicin, gemcitabine, paclitaxel, and topotecan in combination with Dong Quai. The IC_{50} of each anticancer agent was administered alone and in combination with selected clinically relevant concentration 0.86 mg/mL of Dong Quai. Each drug combination was completed in quadruplicate and the experiments were repeated in triplicate. The assessment of drug synergy was done using an isobolar method and the interaction index for each combination for each cell line was calculated. The index, denoted by γ is defined by the isobolar relation a/A+b/B=γ where A and B are the doses of drug A (alone) and B (alone), respectively, that give the specified effect and (a,b) is the combination dose that produces this effect level. For each combination mean dose values were used in the equation to determine the mean interaction index. The quantities in this equation were obtained from the dose response curves of drugs used in each combination. If γ=1 the interaction is additive; if γ is less than one it is super-additive (synergistic), and if γ is greater than one it is sub-additive (antagonistic) [14]. After determining Interaction Index in each cell line, the mean Interaction Index for panel of cancer cell lines was determined for each drug.

Results

At the estimated clinically relevant concentration of 0.86 mg/mL, Dong Quai did not demonstrate inhibition of the CYP450 2C8, 2C9, 3A4, or 2D6 pathways in *in vitro* CYP450 inhibition assays. The *ex vivo* hepatic metabolism induction assay, using cryo preserved human hepatocytes, after treatment with Dong Quai 0.86 mg/mL induction of CYP450 2C8, 2C9, 2D6 and 3A4 was observed. For instance, at 2 hours, Dong Quai caused 27.18% induction compared with 25.17% induction

Cytochrome P450 isoenyzme	Inhibitor	Inducer
3A4	–	+
2C8	–	+
2C9	–	+
2D6	–	+

In the *in vitro* inhibition assay, Dong Quai didn't exhibit any inhibition effect on any of the four isoenzymes. However in the *ex vivo* human hepatocytes, Dong Quai induced CYP3A4, 2C9, 2C8 and 2D6 at clinically relevant concentration 0.86 mg/mL and was comparable to the control inducing agent, rifampin.

Table 3: Result of inhibition assay and induction assay results.

Cell lines	Dong Quai + Carboplatin	Dong Quai + Doxorubicin	Dong Quai + Gemcitabine	Dong Quai + Paclitaxel	Dong Quai + Topotecan
TOV-112D	2.44 Antagonistic	1.77 Antagonistic	0.97 Synergistic	2.02 Antagonistic	1.91 Antagonistic
$SKOV_3$	0.94 Synergistic	2.25 Antagonistic	1.48 Antagonistic	2.25 Antagonistic	2.25 Antagonistic
HeLa	1.74 Antagonistic	2.41 Antagonistic	2.93 Antagonistic	0.92 Synergistic	1.41 Antagonistic
SiHa	1.99 Antagonistic	1.48 Antagonistic	1.10 Antagonistic	1.27 Antagonistic	1.30 Antagonistic
Ishikawa	2.13 Antagonistic	2.88 Antagonistic	1.46 Antagonistic	2.19 Antagonistic	2.00 Antagonistic
HEC-1B	0.82 Synergistic	1.40 Antagonistic	1.18 Antagonistic	1.00 Additive	0.87 Synergistic
MCF-7	0.76 Synergistic	1.52 Antagonistic	1.41 Antagonistic	1.30 Antagonistic	1.65 Antagonistic
NCI-H23	1.48 Antagonistic	1.48 Antagonistic	2.05 Antagonistic	0.92 Synergistic	1.36 Antagonistic
SUMMARY Mean ± SD	**1.54 ± 0.64 Antagonistic**	**1.90 ± 0.55 Antagonistic**	**1.57 ± 0.64 Antagonistic**	**1.48 ± 0.58 Antagonistic**	**1.59 ± 0.45 Antagonistic**

Interaction Index γ=a/A+b/B. Eight human cancer cell lines were treated with the combination of a clinically relevant concentration of Dong Quai and IC_{50} concentrations of five chemotherapy drugs. The combination evaluated demonstrated decreased growth inhibitory activity for all combinations in all cell lines. The Interaction Index γ, was calculated for each cell line and each combination and then summarized with mean Interaction Index. In summary, overall Dong Quai was antagonistic in combination with the chemotherapy agents evaluated.

Table 4: Summary of the Mean Interaction Index of Dong Quai with five chemotherapy drugs.

Drug	Substrate	Inhibitor	Inducer
Cyclophosphamide	2B6		
Ifosfamide	3A4		
Paclitaxel	2C8/2C9, 3A4		2C8/2C9
Docetaxel	3A4		
Tamoxifen	2C9, 2D6, 3A4		
Letrozole	2A6/3A4		
Irinotecan	3A4		
Topotecan	3A4		
Vincristine	3A4		
Imatinib	3A4	3A4	
Doxorubicin	3A4	2D6	
Epirubicin	3A4		

The table is a summary of CYP450 metabolism pathways for commonly used chemotherapy agents. A substrate=metabolized by this CYP450 isoenzyme pathway; inhibitor=it will decrease the activity, preventing metabolism via the isoenzyme pathway resulting high parent drug concentrations and potentially increase toxicity; inducer=it will increase the enzyme activity, increasing metabolism via the isoenzyme pathway resulting lower parent drug concentrations and potentially decrease drug activity. Dong Quai may have the potential to induce the metabolism of all the chemotherapy agents listed on this table.

Table 5: CYP450 metabolism pathways of common anticancer agents.

by control rifampicin. Comparison with two treatments generates a p value of 0.52 indicating that there was no significant difference in the induction of 2D6 metabolism with Dong Quai compared with that seen with known control inducer, rifampicin. The results of inhibition assay and induction assay are summarized in Table 3.

The IC_{50} for single agent activity was not achievable. The IC_{25} for Dong Quai single agent activity ranged from 0.39 mg/mL to 4.48 mg/mL in each of the eight human cancer cell lines which is above the estimated clinically relevant concentration of 0.86 mg/mL indicating that Dong Quai did not independently exert cytotoxic activity in the cancer cell lines. The combination of clinically relevant concentration of Dong Quai (0.86 mg/mL) with IC_{50} concentration of each of the five chemotherapy drugs evaluated including: carboplatin, doxorubicin, gemcitabine, paclitaxel, and topotecan demonstrated decreased growth inhibitory activity for all combinations in all cell lines. The interaction index for each combination of Dong Quai with chemotherapy drug was calculated for each cell line as described in materials and methods. While a few combinations demonstrated synergy in selected cell lines, overall Dong Quai have interaction index ranging from 1.48-1.9, suggesting antagonistic activity with all the chemotherapy agents evaluated in the panel of ER+ human cancer cell lines. The interaction index data is summarized in Table 4.

Discussion

While exposure to Dong Quai was not associated with inhibition of any of the CYP450 isoenzyme hepatic metabolism pathways, induction of CYP4502C9, 2C8, 2D6 and 3A4 hepatic metabolism pathways was observed after exposure to estimated clinically relevant concentration of Dong Quai 0.86 mg/dL. This indicates that Dong Quai may have a potential for drug interactions with drugs metabolized by these CYP450 isoenzyme hepatic metabolism pathways. Table 5 provides a list of commonly used chemotherapy agents that may interact with Dong Quai due to CYP450 induction of 2C9, 2C8, 2D6 and 3A4. This would be important to consider when make recommendations to combine use of Dong qui with other medications. Furthermore, the results of the growth inhibition assays indicated that Dong Quai had an overall antagonistic interaction with the cytotoxic activity standard chemotherapy agents in the panel of eight human cancer cell lines. While in a few cell lines there was observation of synergistic activity, this study was focusing on safety for making general recommendations for use of Dong Quai in combination with chemotherapy. Since tumors have inherent variability, a conservative approach to draw a conclusion for activity was based on antagonistic, additive or synergistic activity in the majority (four or more) of cell lines evaluated as well as determination the mean Interaction Index value. These finding are important because the combination of a potential drug-drug interaction involving CYP450 and antagonistic effect with standard chemotherapy agents suggests that Dong Quai would not be safe to use in cancer patients while receiving chemotherapy.

The findings in our study are consistent with finding from previous pre-clinical studies that used higher doses, what are unlikely

clinically achievable concentrations, of Dong Quai but also observed potential tumor potentiation. For example an *in vitro* study showed that Dong Quai dose-dependently and significantly stimulated the proliferation of MCF-7 cells with a weak estrogen-agonistic activity in the presence of 17 beta-estradiol, as evidenced by the significant suppression by 4-hydroxytamoxifen [9]. While another study found Dong Quai enhanced the proliferation of breast cancer cell line MCF-7 independent of estrogen activity [15]. Thus the antagonistic interaction observed in this study could be attributed to direct cancer cell potentiation and not an interaction directly with the chemotherapy agent itself. However, the metabolism data still suggests potential for drug-herbal interaction as well. A good example to consider confirm metabolism contributing factor is that Dong qui is classified as a "phytoestrogen" and potentiation of growth has only been reported in estrogen-mediated cancer, however this study did observe antagonistic activity in the non-small cell lung cancer cell line as well.

As one of the most commonly used CAM agents for female reproductive function improvement, Dong Quai has become the study of interest of many investigators. Prior to evaluating it as potential herbal/natural alternative to HRT for the management of hormone-mediated symptoms, it is necessary to define the pharmacology profile of Dong Quai in humans. In this study an estimated "achievable concentration" was used to define potential for drug-herbal interactions that assumes 100% bioavailability to give a "highest possible" relevant concentration which was better than arbitrarily selecting a dose to use in the studies. However, this is a limitation of this study because 100% bioavailability is unlikely with any compound/drug. Determining safety is a priority before starting further clinical study design, especially for use in women with estrogen mediated cancer or even strong family history of cancer. Pharmacokinetic study in healthy volunteers is being pursued to confirm the achievable Dong Quai concentration which will give better information for determining if it is a candidate for to be a safe and effective phytoestrogen to manage hormone-related symptoms in women with cancer receiving active chemotherapy treatment.

In conclusion, this data suggests that Dong Quai is an inducer of the CYP450 pathways and also decreased cytotoxic activity of selected chemotherapy. Keeping in spirit of "first does no harm", conservative recommendations are being made at this time based on the pre-clinical data available. However, the metabolism data still suggests potential for drug-herbal interaction as well. Until prospective pharmacology data in humans to determine what systemic plasma concentration of Dong qui is achievable and confirmatory *in vivo* information is available, Dong Quai should be used with caution in patients with cancer receiving chemotherapy.

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