

## Herbal products may alter drug metabolism

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**Background.** Adverse reactions to therapeutic drugs are a significant and increasing cause of death among patients (Burgess, Holman, Satti, 2005). Pharmacological actions of drugs, both beneficial and adverse, are largely dependent on bioavailability, the amount of active chemical that reaches systemic circulation. This is determined by the activity of drug metabolizing enzymes after drug ingestion and before excretion. The role of these enzymes involves detoxication, the conversion of active compounds into inactive metabolites, or toxication, the conversion of inactive compounds into active metabolites, in the case of prodrugs. Cytochrome P450s (CYPs) are a major group of phase-I oxidase enzymes that are primarily found in the liver and intestine. They metabolize xenobiotics, foreign chemicals, by converting them into water-soluble products that are readily excreted from the body. The human enzyme CYP3A4 is responsible for 60% of drug metabolism, notably of chemotherapeutic agents, antidepressants, HIV antivirals, statins and oral contraceptives (Izzo, 2004). As a result of its variety of substrates, changes in expression of the CYP3A4 gene can influence the dose of many drugs required to be effective, the duration of their action, and rate of excretion of many drugs and their metabolites. Despite its crucial role in our body, CYP3A4 gene expression is not stable, but is subject to xenobiotic-mediated regulation via the transcription factor pregnane X receptor (PXR). Lack of PXR ligand specificity results in a wide range of chemicals, such as the pharmaceutical rifampicin, that are capable of regulating CYP3A4 gene transcription and subsequent metabolic rates. As a result of the importance of CYP3A4 metabolism, many compounds have been investigated for their effects on CYP3A4 activity. Several, such as

grapefruit juice and St. John's wort (SJW), induce or inhibit enzyme activity and have been observed to alter the pharmacokinetics of pharmaceutical drugs in patients. In cases of warfarin and cyclosporine therapies, SJW was implicated in life-threatening complications (Bailey, et al., 2005) and has reduced systemic concentrations of HIV antivirals indinavir and saquinavir by 43% (Piscitelli, et al., 2000). Given the biological activities of herbal and botanical products, they may pose a similar risk of drug interactions and may affect the success of drug therapies. The use of herbal products in conjunction with drug therapies is increasingly prevalent among patients on cancer and HIV treatment (de Jong, et al., 2007). Since there is a lack of knowledge about the biological effects of herbal products, investigations may lead to relevant applications in clinical human health.

**Purpose, Hypothesis.** The purpose of this investigation was to determine the impact of herbal products on hepatic PXR-mediated CYP3A4 transcription. It was hypothesized that popular immune stimulating herbal products associated with drug therapies would regulate CYP3A4 transcription in a dose-dependent manner.

**Procedure.** A dual luciferase transcription assay was used. Plasmids containing the CYP3A4 proximal promoter adjacent to the firefly luciferase gene enable the detection of CYP3A4 transcription levels based upon luminescence of cell lysates after treatment with test compounds.

1) *Cell culture and transfection.* A human hepatocellular carcinoma cell line (HepG2) was cultured in 24 well plates for 24 hours. Reporter plasmids containing either the human PXR sequence, the Renilla sequence (secondary luciferase as control plasmid) or the CYP3A4-luciferase sequence were transfected into cell cultures using a lipofectamine 2000 reagent for 24 hours.

2) *Preparation and Treatment.* Treatments were dissolved into solvents DMSO (dimethyl sulfoxide), ethanol or water and diluted to the appropriate concentration. The cells were treated for 48 hours with treatments (listed below).

3) *Luciferase detection.* The cells were washed with phosphate buffered saline (PBS), then treated with a passive lysis buffer, and stored below -80 °C. Luciferase activity of cell lysates was determined by two separate luminescent reactions involving detection of luciferase activity with luciferin and detection of Renilla activity with Stop n' Glow. The samples were analyzed using a Fluostar Optima machine, detecting the amount of light emitted from each reaction. The luciferase activity was normalized to the Renilla (control plasmid) activity to account for transfection efficiency and cell numbers. Results were based upon the increase of light emitted from the treated samples compared to the controls.

4) *Statistical analysis.* Data was represented as the population mean normalized to the control. Graphs showed standard error and an analysis of variance (ANOVA) test was performed to compare control to treatment populations and determine the p-value. The significance: \* (significant;  $P < 0.05$ ), \*\*, (very significant;  $P < 0.01$ ) or \*\*\* (extremely significant;  $P < 0.0001$ ) was then assigned.

The treatments that were investigated included compounds that were established CYP3A4 inducers: rifampicin, phenobarbital, RU486, SJW (*Hypericum perforatum*) and vitamin D, or novel test compounds: Cold-fX (*Panax quiquefolius*), Five-Flower Formula, consisting of Cherry plum (*Prunus cerasifera*), Climatis (*C. Vitalba*), Impatiens (*I. glandulifera*), Rock rose (*Helianthemum nummularium*) and Star of Bethlehem (*Ornithogalum umbellatum*), ginseng (*Panax ginseng*), Essiac tea, a formula of burdock root (*Arctium lappa*), sheep sorrel leaves (*Rumex acetosella*), slippery elm bark (*Ulmus rubra*) and Indian rhubarb root (*Rheum officinale*),

Siberian ginseng (*Eleutherococcus senticosus*), astragalus (*Astragalus membranaceus*), or echinacea (*Echinacea purpurea*). These are some of the most widely used immune-stimulating herbal products used by patients on cancer and HIV treatment (Balch, 2002).

**Results.** Results demonstrated the regulatory potential of herbal products in PXR-mediated CYP3A4 transcription. The following substances known to increase CYP3A4 transcription, rifampicin, RU486, SJW and vitamin D, all induced luciferase production, showing the positive relationship between the two. Ginseng, Essiac tea, Five-Flower Formula, Siberian ginseng, and echinacea all increased luciferase activity by at least 2-fold in a dose dependent manner. Notably, ginseng, echinacea and Siberian ginseng's effects were comparable to SJW. Combinations of Essiac and echinacea increased CYP3A4 transcription higher than either treatment alone, while ginseng appeared to inhibit inductive effects of high dose echinacea.

**Conclusions, Applications.** Results from luciferase assays have been shown, by Raucy (2003) to reflect CYP3A4 regulation in human hepatocytes, and therefore the observations in this experiment likely represent effects that would occur in humans. Luciferase induction observed in this investigation suggests that echinacea and Siberian ginseng may also alter drug bioavailability to the same degree and by the same mechanism as SJW. A multitude of bioactive constituents have been documented in SJW, which may explain the observations that SJW inhibits CYP3A4 activity after a short exposure time, yet induces CYP3A4 activity after a longer exposure (Pal, Mitra, 2005). This may be the case in the observations that echinacea induced or inhibited luciferase concentrations dose-dependently.

Results from this investigation propose PXR-mediated CYP3A4 induction may be the mechanism for interactions between ginseng and warfarin described by Izzo (2004). As well, the identification of Essiac as an inducer of CYP3A4 is important since many cancer patients may

use it in conjunction with chemotherapy drugs, which could induce drug toxicity or decrease effectiveness. While luciferase induction was observed mainly for the chemicals tested in the presence of PXR, compounds may also interact with CYP3A4 by other inhibitory mechanisms, such as enzyme neutralization, inter-substrate competition and allosteric regulation of PXR. Allosteric regulation may be the mechanism by which ginseng induced luciferase independently, yet in combination with echinacea, reduced echinacea-mediated luciferase induction.

Currently, 40% of chemotherapeutic agents are derived from plants, and knowledge of organic molecules' affinity for PXR binding may help predict drug-drug interactions. Determining the PXR ligand specificity may be significant, as the receptor has been described as a "master regulator" in xenobiotic function (Mu, Stephenson, 2005). Knowledge of this pathway could lead to more efficient drugs with more precise dosages or the development of products without bioactive compounds, such as furanocoumarin-free grapefruit juice (Paine, Widmer, Hart, 2006). Herbal product regulation of CYP3A4 function may have broader health implications than drug reactions alone, as endocrine disruption has been observed from altered estrogen metabolism (a substrate of CYP3A4), leading to predisposition of osteoporosis (Kang & Park, 2009) or blood clotting (Grande & Mendelez, 2009). Such reactions also convert pro-carcinogens, such as aromatic hydrocarbons, into a toxic form (Perantoni, 2006). An increase or inhibition of CYP function may cause toxins to accumulate and may result in hepatic injury. Based upon the importance of CYP3A4 function in drug therapies and human health, further investigation is needed to identify and raise awareness about other herbal products altering CYP activity.

**Acknowledgements.** I would like to thank Kathleen Nichols for her continuing mentoring, guidance and expertise, as well as Dr. Gordon Kirby, University of Guelph, for the use of his lab.

## Appendix

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