Hepatic model helps to predict clinically-relevant botanical-drug interactions

Use of botanical-based dietary supplements is increasing among people of all ages and in most geographies. Most of these populations have ready access to conventional medications, with significant polypharmacy observed in older adults.

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The scientific literature is replete with reports of botanical extracts and/or their constituents as potent inhibitors of drug-metabolising enzymes and transporters. Unfortunately, most of these studies use simplistic *in vitro* screening-based systems without follow-up in more physiologically-relevant models. Additionally, the potential for botanical extracts to induce metabolic enzymes and/or transporters is rarely studied, particularly in *in vitro* systems. Thus, in the rare instances when clinical studies are conducted to confirm botanical-drug interaction (BDI) potential, there is usually a poor correlation between *in vitro* studies and clinically-relevant changes in the pharmacokinetics of drugs under study.

New paradigms are necessary to assess BDIs. One such method uses sandwich-cultured human hepatocytes and an *in vitro* clearance approach that treats the complex botanical mixture as a single entity, regardless of the constituent profile. This *in vitro* approach captures the major hepatic drug clearance pathways and provides predictions that are easily translatable to the clinic. Thus, it identifies potentially significant and insignificant interactions. By studying complex mixtures using this method it is possible to evaluate the overall net effect on drug clearance mechanisms and capture synergism and additive effects that occur among the constituents within these complex mixtures – effects that may not be observed with a traditional deconstructionist approach.

This case study will show how the sandwich-cultured human hepatocytes model was used to predict clinically-relevant BDIs using *Schisandra spp*. It also potentially demonstrates applications for the sandwich-cultured human hepatocytes model in evaluating BDIs for other herbal extracts and for identifying hepatotoxicity endpoints.

Introduction

Rising sales of botanical supplements leads to increasing concern about BDIs

In 2017, botanical-based dietary supplements surpassed $8 billion in sales, and showed the greatest growth in 15 years[1]. The consistent increase in supplement sales during the past decade reflects consumers' interest in alternative health approaches (eg Ayurvedic herbs, traditional Chinese medicine), new formulation options (enhanced absorption technologies) and culinary botanicals with general health and nutrition benefits.

The increase in dietary supplement popularity, coupled with the already heavy use of prescription drugs in the US, highlights the importance of studying BDI potential[2]. Further adding to the risk of concomitant use interactions is the documented lack of dialogue between patients and healthcare

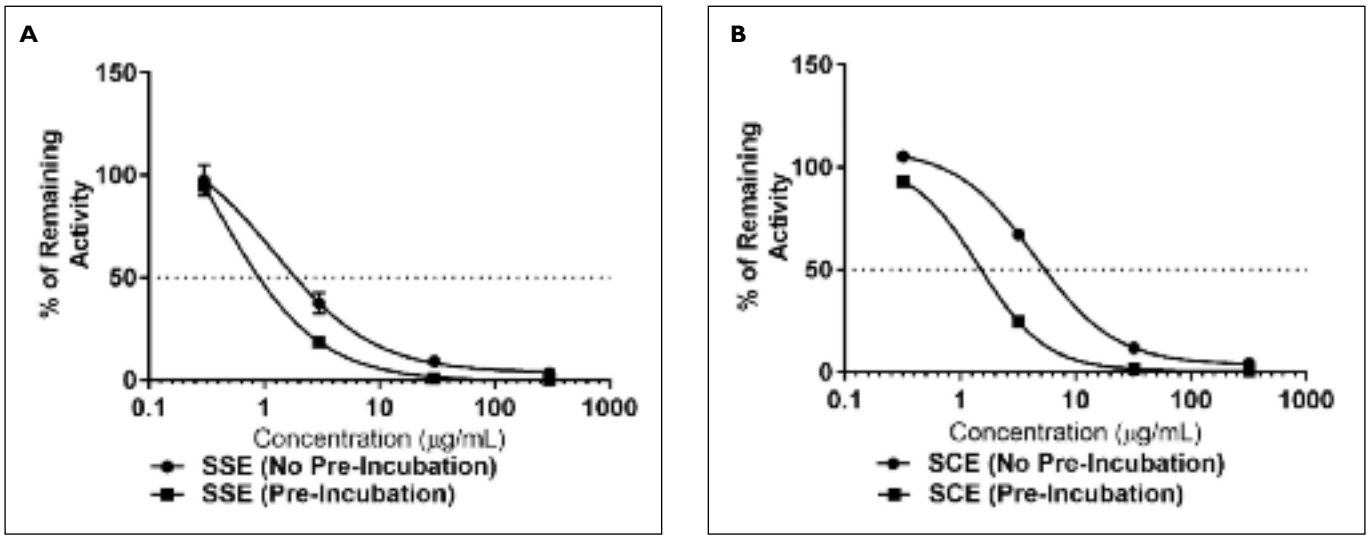

Figure 1: Inhibition of CYP3A4/5 by SSE (A) or SCE (B), without preincubation (●) and with preincubation (■)

professionals about dietary supplement use, despite media attention warning about potential BDIs. We believe that providing information on potential BDIs can facilitate informed decision-making by consumers and healthcare providers.

Conventional methods to investigate BDIs are inadequate

The scientific literature includes numerous reports of the potential of botanical extracts and/or specific phytochemical constituents to inhibit cytochromes P450 enzymes (CYP450). These studies are typically done with isolated *in vitro* systems such as liver microsomes; thus, CYP450 inhibition is the only interaction mechanism studied. There are also reports of quite potent inhibition of CYP450s, particularly when individual phytochemical constituents are used in these assays. The interaction of botanical extracts with transporter proteins is also studied using *in vitro* systems such as Caco-2 cells. Unfortunately, follow-on studies using more physiologically-relevant *in vitro* models and/or human clinical studies are rarely conducted. There is also a paucity of data on the ability of botanical extracts or phytochemical constituents to induce CYP450 enzymes.

For the few botanical extracts where interaction potential has been studied in both *in vitro* and clinical studies, the correlation has been poor[3]. In a recent review, Sprouse and van Breemen compared results from available preclinical data (largely *in vitro*) with clinical results across a number of botanicals or phytochemical complexes[3]. Only in a few examples, such as echinacea, garlic, goldenseal and St John's wort, do *in vitro* studies predict similar findings on CYP450s to clinical studies. The *in vitro* systems used in these studies are even less likely to predict clinically-meaningful changes.

The aforementioned review acknowledges that there are a number of explanations for the poor correlation of *in vitro* data to clinical findings. It is difficult to compare botanical materials across studies to ensure similarity of test article, and the solubility of the complex mixtures represented by botanical extracts can be challenging to study in certain *in vitro* systems. The authors also mention that the *in vitro* model(s) used, and the lack of including CYP450 induction in addition to inhibition assessments, can impact the ability to predict clinical relevance.

An additional complication with BDI predictions based on these simplistic screening-level *in vitro* studies is that the data are incorporated into safety-related databases and publicly-available websites without critical review. This may cause undue concern for consumers and healthcare professionals regarding the concomitant use of certain dietary supplements with prescription medicines. Likewise, even scientific reports, such as the review by Sprouse and van Breemen, can inappropriately impugn the utility of *in vitro* metabolic and transporter systems to predict if and when clinical follow-up studies are warranted.

Our strategy: investigate a known BDI by treating a botanical mixture as a single entity

Due to the above-mentioned challenges, we wanted to develop an alternative approach for studying BDIs that would improve the ability of *in vitro* models to predict clinically-meaningful changes in

drug pharmacokinetics. We had the following goals: use a methodology that could treat a complex botanical mixture as a single entity, regardless of specific phytochemical constituents; utilise an *in vitro* tool that can study synergistic or competitive effects between phytochemical constituents; and design an experimental strategy that captures major clinically-relevant hepatic pathways and data output and makes direct predictions regarding clinical data. Thus, we chose to look at metabolic clearance changes of drugs using sandwich-cultured human hepatocytes exposed to botanical extracts. Our proof-of-concept study involved the use of *Schisandra spp*. with available *in vitro* and *in vivo* published data.

Schisandra spp. have a wide range of purported pharmacological activities including antioxidant, anti-inflammatory, antibacterial and cardio-protection. *S. chinensis* is regulated as a dietary supplement in most geographies and *S. sphenanthera* is a marketed drug in China. In *in vitro* studies conducted by Iwata et al, various phytochemical constituents of *S. chinensis* were tested for the ability to inhibit CYP450 activities[4]. The Gomisin class of constituents was shown to inhibit CYP3A4 with IC_{50} values as low as 0.257μM. The IC_{50} values observed were similar in potency to the known CYP3A4 inhibitor; ketoconazole. To date, there have been no clinical drug interaction studies conducted with *S. chinensis*. The related species, *S. sphenanthera*, has been studied in at least two clinical drug interaction studies. Xin et al studied the effects of *S. sphenanthera* extract on the pharmacokinetics of tacrolimus in healthy volunteers, since this combination is often used in renal and liver transplant recipients in China[5]. The AUC of tacrolimus increased on average 164.2% following administration of *S. sphenanthera*. Similarly, these same researchers conducted a study in which *S. sphenanthera* was co-administered with midazolam[6]. In this study, AUC of midazolam was increased 119.4% after administration with *S. sphenanthera*.

Based on these findings, we utilised the sandwich-cultured human hepatocytes *in vitro* model to recapitulate the clinical findings of co-administration of *S. sphenanthera* with midazolam. Furthermore, we wanted to conduct similar *in vitro* studies with co-treatments of *S. chinensis* and midazolam. Successful correlation of *in vitro* to *in vivo* findings between midazolam and *S. sphenanthera* would provide confidence in extrapolating

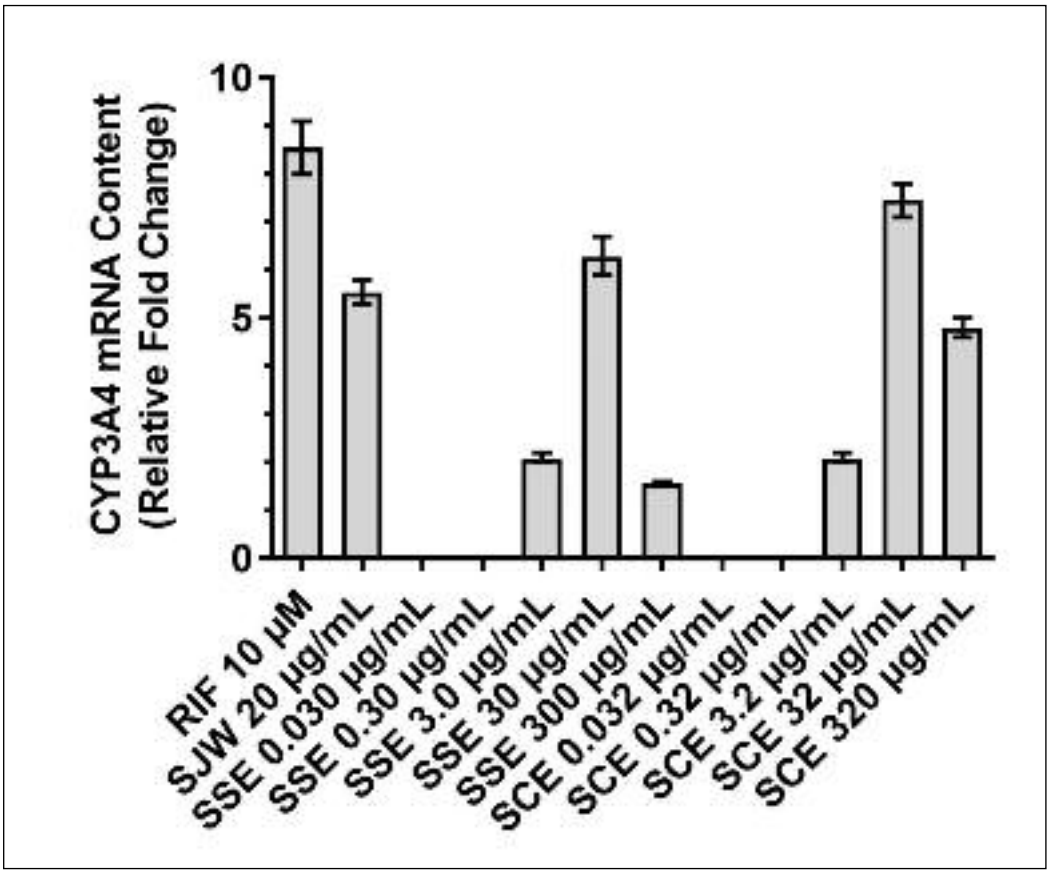

Figure 2
Relative-fold change of CYP3A4 mRNA content in sandwich-cultured human hepatocytes following 72 hours of treatment with RIF (10mM), SJW (20mg/ml), SSE or SCE. Error bars represent 95% confidence intervals

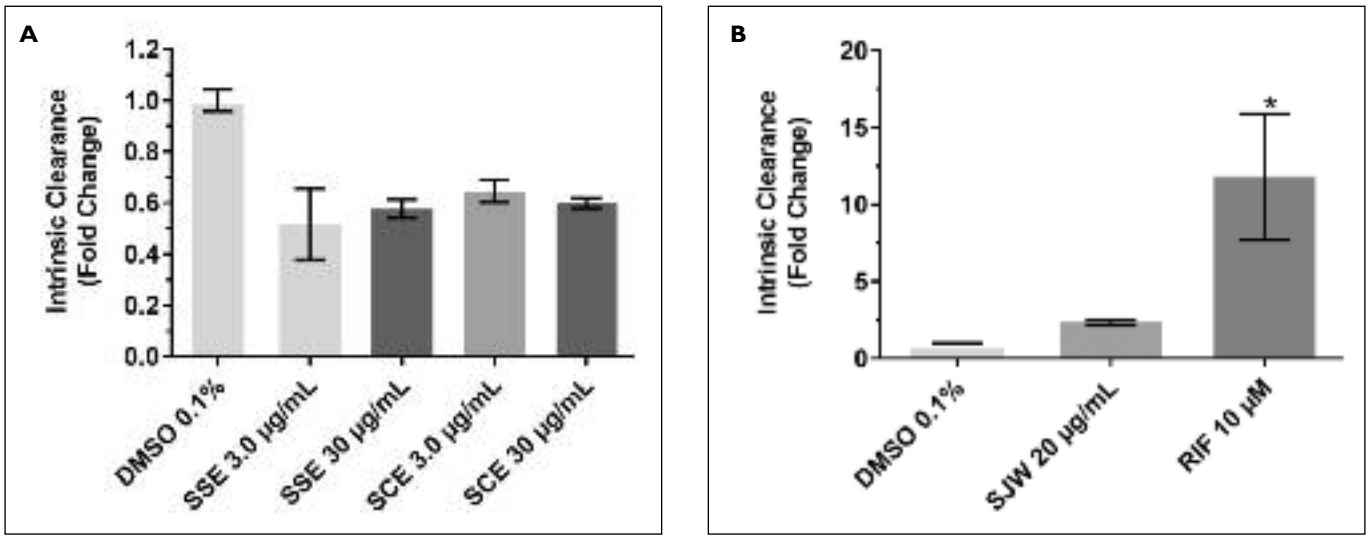

Figure 3: Intrinsic clearance of MDZ was calculated from linear regression analysis of the percentage of parent remaining (log transformed) versus time profile to estimate the elimination rate. *P value ≤0.05 when compared with solvent control

in vitro findings of *S. chinensis* to predict potential clinically-relevant interactions.

Sandwich-cultured Transporter Certified™ hepatocytes as a model for studying clinically-relevant botanical-drug interactions

Transporter Certified™ hepatocytes in sandwich culture provide a fully-integrated hepatic cell system that maintains drug clearance pathways (metabolism and transport) and key regulatory pathways necessary for quantitative assessments of BDI potential[7-8]. Because the system integrates biliary excretion, intracellular concentrations are likely more physiologically-relevant resulting in more accurate recapitulation of *in vivo* conditions compared to other systems. Other hepatocyte systems (eg conventional monolayer) do not support biliary excretion resulting in higher intracellular concentrations likely biasing the ultimate cellular outcome (eg toxicity). When one considers the use of liver microsomes for BDI assessment, which do not account for drug transport or adaptive response (eg induction), it becomes readily clear how results from such simplistic systems could under- or over-predict clinically-relevant risk. Thus, it is not surprising that poor *in vitro* to *in vivo* correlations have been observed in the majority of *in vitro* studies conducted with botanical extracts.

Proof-of-concept study design

We evaluated the effectiveness of the sandwich-cultured human hepatocytes model in predicting *in vivo* BDI outcomes using an intrinsic clearance approach. Additional mechanistic studies focused on inhibition and induction of CYP3A4 were performed to asses specific BDI following *Schisandra spp*. exposure[9].

When designing BDI studies, quantification of at least the major phytochemical constituents is helpful in setting dose concentrations for use in *in vitro* studies. Unfortunately, there is rarely plasma concentration data on extract constituents so it is particularly important in these studies to include a wide range of dosing concentrations. As mentioned above, the intracellular concentration of a compound is responsible for the ultimate cellular outcome(s).

Results

Mechanistic inhibition studies in sandwich-cultured human hepatocytes demonstrated that *S. chinensis* and *S. sphenanthera* inhibited the rate of hydroxymidazolam formation (CYP3A4/5 activity) from midazolam in a concentration and time-dependent manner (**Figure 1 A-B**). Pre-incubation with either extract increased inhibition potency (eg IC_{50}) ≥3-fold indicative of time-dependent inhibition[9].

Induction assessment in sandwich-cultured human hepatocytes demonstrated *S. chinensis* and *S. sphenanthera* induced CYP3A4 mRNA content in a dose-related manner following 72 hours of exposure (**Figure 2**). Induction responses stimulated by SSE (30mg/ml) and SCE (32mg/ml) exposure were greater than the response produced by St John's wort (SJW) treatment, a well-established

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herbal inducer of CYP3A4, and were ≥73% of the rifampicin (RIF) treatment, a prototypical inducer of CYP3A4[9].

To predict the overall clinical effect, evaluation of midazolam intrinsic clearance following 72 hours of exposure demonstrated *S. chinensis* and *S. sphenanthera* both reduced midazolam clearance 35-40% and 42-48%, respectively (**Figure 3 A-B**). Our *in vitro S. sphenanthera* results were in remarkable agreement with a 52% decrease in clearance observed in clinical midazolam interaction studies. Additionally, our *in vitro* results were in good agreement with clinical-interaction studies of well-known perpetrators of *in vivo* drug interactions, SJW and RIF. In clinical drug-interaction studies, SJW and RIF increased midazolam clearance 2.0-fold and 24-fold, correspondingly[10,11].

Conclusion

Our retrospective case study demonstrated that an intrinsic clearance approach in sandwich-cultured human hepatocytes was feasible to evaluate net effect and relative strength of BDIs. We believe our 'net-effect' approach is an appropriate strategy to screen complex mixtures for drug interactions and can effectively predict *in vivo* BDIs at a much lower cost than clinical studies. DDW

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