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Review Article

IN VITRO EVALUATION OF HERB-DRUG INTERACTIONS: A REVIEW

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ABSTRACT

Herbal medicinal products are used worldwide and have become important part of clinical medicine. Today with the increasing level of consumer acceptance, safety issues related to herbal drugs continue to be ignored by the public, neglected by manufacturers as well as under-researched by the medical professionals. Users of herbal medicines tend to believe that herbals are inherently safe and thus concomitantly use self-prescribed herbal and prescribed synthetic drugs. Thus, there is a high possibility of interactions between herbal and synthetic drugs. Interactions of several commonly used herbal medicines such as Gingko biloba, St. John's wort with therapeutic drugs including Warfarine, Midazolam, and Nifedipine in humans have been reported. The combined use of herbs and drugs has increased the possibility of pharmacokinetic and pharmacodynamic interactions. Herb drug interactions (HDI) involve drug metabolizing enzymes Cytochrome P450 (CYP450) and drug transporters P-glycoprotein (P-gp). As the herb drug interactions significantly alter pharmacokinetic and pharmacodynamic properties of administered drugs, the drugs interacting with herbal medicines need to identified by appropriate *in vitro* and *in vivo* methods. In this review, we have summarized different mechanisms of herb-drug interactions mediated by CYPs and P-gp and *in vitro* methods to assess them.

Keywords: Self- prescribed, Herb- drug interactions, CYP450, P-gp, *In vitro*.

INTRODUCTION

Herbal medicine usage has increased throughout the world for the treatment of various disorders. General perception is that herbals are inherently safe, hence sometimes they are self prescribed with the prescribed synthetic drugs without knowledge of the medical practitioner. When herbal medicines and conventional allopathic drugs (prescription or non-prescription medications) are used together, they can interact in our body causing changes in the way the herbs and/or the drugs work. Such changes are called 'Herb-drug interactions'. They are harmless but sometimes really prove to be fatal [1].

The nature of herb-drug interactions is not a chemical interaction between a drug and an herb component to produce something toxic. The interaction involves an herb component causes either an increase or decrease in the amount of drug in the blood stream[1,2]. A decrease in the amount of drug occurs when herb components binds to the drug and preventing it from getting into the blood stream from the gastrointestinal tract or by stimulating the production and activity of enzyme that degrade the drug and prepare it for elimination from the body. This decrease in drug dosage could make the drug ineffective. An increase in the drug dosage could occur when an herb component aids absorption of the drug or inhibits the enzymes that break down the drug and prepare it for elimination. An increase in drug dosage could make it reach above maximum safe levels that may produce side effects or toxicity.

MECHANISM OF HERB-DRUG INTERACTIONS (HDI'S)

Herb-drug interactions are generally characterized by pharmacokinetic and pharmacodynamic interactions. Pharmacokinetic interactions occur when herbal formulation changes absorption, distribution, metabolism, protein binding or excretion of a drug. It involves drug metabolizing enzymes (CYP450) and drug transporters (P-gp). Some drugs are substrates for both CYP450 and P-gp thus higher probability that it will interact with herbal products. The metabolism of any drug involves two parts. Phase I reactions (biotransformation) include oxidation, hydroxylation, reduction, and hydrolysis. In these enzymatic reactions, a new functional group is introduced into the substrate molecule and an existing functional group is modified or a functional group acceptor site for phase II transfer reactions is exposed, thus making the xenobiotic more polar and therefore, more readily excreted[5,6]. This phase is carried out mainly by cytochrome P450 isoenzymes which are present in the liver these may be induced or inhibited. Phase II reactions (conjugation) are enzymatic syntheses whereby a functional group such as alcohol, phenol, or amine is masked by the addition of a new group such as acetyl, sulfate, glucuronic acid or certain amino acids which further increases the polarity of the drug or xenobiotic. Most substances undergo both phase I and phase II reactions sequentially [7, 8].

The other major mediator of HDIs is P-glycoprotein (P-gp) transporters which are membrane bound proteins regulating the influx and efflux of drugs across the plasma membrane. Pharmacodynamic interactions include mutual action of herb and drug inside the body. Pharmacodynamic interactions are difficult to predict or prevent [9]. When herb and drug are taken simultaneously it may show synergistic or antagonistic effects (Fig.1).

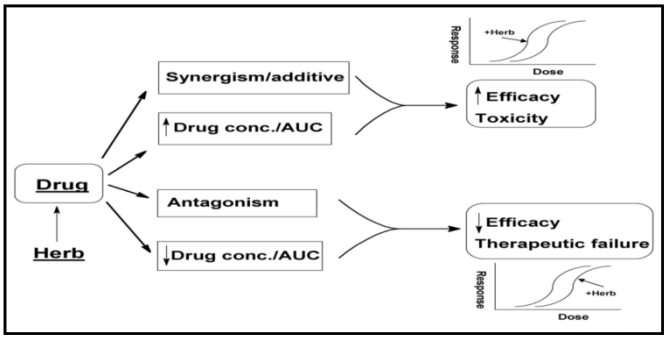

Fig. 1: Possible outcomes when a drug interacts with herbal medicine [17]

METHODS TO ASSESS HERB-DRUG INTERACTION

To avoid or minimize toxic herb–drug interactions it is important to identify drugs that can interact with herbs using proper *in vitro* and *in vivo* models in the early stages of drug development. *In vitro* methods using liver or intestinal microsomes, cytosols, human hepatocytes, transfected cell lines, tumor derived cells have been widely used. *In vivo* methods have been reported in transgenic or humanized animals and in healthy human individuals. Each *in vitro* and *in vivo* systems has advantages and limitations (Table 1). *In vitro* methods are preferred over *in vivo* as they are less complex, easy to carry out, reliable and provides mechanistic information. It also gives information on specific CYP isoform, induction or inhibition, metabolite profile and existence of herb-drug interactions [10].

Table 1: Comparision of study methods available for HDI

Methods	Advantages	Limitations	Ref.
In vitro studies	Easy to perform, good for high throughput screenings, results are closer to human.	Poor reproducibility of results, poor correlation to clinical situation.	[10,11]
In vivo studies	Concentration and bioavailability of active components are taken into consideration. Differences in biology and pharmacokinetics of species.	Results are difficult to interpret, variation in species, use of variable dosage forms.	[10,11]
Case reports	Providing information on HDI.	Poor statistical values.	[6]
Human studies	Extrapolative data on interactions	Expensive, genetic variation in enzyme activity.	[9,10]

Table 2: Methods for inhibition of CYP

Methods	Mechanism	Advantages	Disadvantages	Ref.
Fluorescence (high throughput)	Pro-fluorescent substrate metabolized to fluorescent product.	Fast, sensitive, cost effective, measures enzyme activity.	Recombinant enzymes needed as probes.	[19,20,34]
Luminescence (high throughput)	Pro-luminescent substrate metabolized to luminescent product.	Very sensitive	Work-up reagent required. Recombinant enzymes needed.	[25,27]
Radiometric (low throughput)	Release of radiolabel on metabolism of substrate	Fast and accurate.	Large amount of radiolabelled substance used.	[19,20]
LC_MS/MS (Medium throughput)	Standard method for all interaction assessment.	Sensitive and specific.	Expensive	
CYP450-CO complex assay	CYP450 microsomes quantitated using characteristic absorbance peak at 450nm	Simple, less laborious, reliable, reproducible	Less sensitive	[28,30]

IN VITRO METHDOLOGIES

Drug metabolizing enzymes: CYP 450 assays

The CYP450 system is a family of heme based enzymes located in hepatocytes, enterocytes and smooth endoplasmic reticulum. *In vitro* studies with human/rat liver microsomes are conducted to evaluate ability of an herb-drug moiety to inhibit CYP1A2, CYP2D6, CYP2C8, CYP2C19 and CYP3A4. Measurement of CYP inhibition is performed by analysing inhibition of substrate metabolism. Herbs may inhibit CYP by direct competitive inhibition (reversible), non-competitive inhibition (irreversible) and mechanism-based inhibition. In reversible inhibition there is direct competition for the binding site on a CYP enzyme between the substrate and an inhibitor. Non-competitive inhibition is caused by the binding of herbal constituents containing electrophilic groups to the heme portion of CYP. The mechanism-based inhibition of CYP is due to the formation of a complex between herbal metabolite with CYP. The methods for measurement of CYP inhibition are fluorescence, luminescence, radiometric and liquid chromatography-tandem mass spectroscopy (LC-MS/MS). They are also categorized as high, medium and low throughput methods [19, 20] (Table 2).

Fluorescence and luminescence based method are high throughput methods in which pro-fluorescent or pro-luminescent substrate is metabolised to fluorescent or luminescent product. In this method CYP450 inhibition kits are used which consists of 96 well microtiter plate, NADPH (Nicotinamide adenine dinucleotide phosphate) reduced form, Tris phosphate buffer, Dibenzyl fluorescein (DBF) which acts as a flurogenic substrate and stop reagent (acetonitrile). Drugs like Ketoconazole and quinidine are inhibitors used as a positive control. Herbal powder or individual plant material under study are extracted by using suitable extraction technique and standardized using various chromatographic techniques used like reverse phase-high performance thin layer chromatography (RP-HPTLC), Liquid chromatography-mass spectrometry (LC-MS). The assay is performed in duplicate. Control wells and blank wells are maintained. Conrol wells contain extracted sample, tris buffer, DBF, NADPH, water and corresponding CYP enzymes.

Blank wells contain all these except test sample. All the enzymes are freshly thaw before adding into the wells. To initiate the reaction NADPH regeneration system is added in to the wells. This system contains $NADP^+$, glucose-6-phosphate, glucose-6-phosphate dehydrogenase and $MgCl_2$. Plates are incubated and reactions are stopped by adding stop reagent and evaluated for quenching and intrinsic fluorescence of the test components after 30 minutes. The values are recorded using fluorescence microplate reader (Perkin Elmer model). Rate of reaction is determined by % change in fluorescence signal. Percentage inhibition and Inhibition concentration (IC_{50}) values are determined [25,27].

Percentage inhibition = 100 – {(Signal of well–Blank)} × 100/ (Solvent control – Blank)

IC_{50} = {(50 – LP) × (HC – LC) + LC} / (HP – LP)

LP = Low percentage of inhibition, HP = High percentage of inhibition, LC = Low concentration, HC = High concentration

Low throughput methods like radiometric and LC-MS/MS are also used individually or in combination. For eg. Chromatographic method combined with radiometric method for quantification of catapol a Chinese herbal medicine. LC-MS/MS based CYP inhibition methods are also reported. Substrates are incubated with the CYP enzymes and resulting metabolites are qunantified with LC-MS/MS. For eg. Evaluation of HDI during intestinal metabolism and absorption of baicalein flavones isolated from Chinese herb [27].

Cytochrome P450 –Carbon monoxide (CO) complex assay

It is a simple microplate assay method. Assay is performed to determine inhibitory potential of the selected herbal formulation and it's individual bioactive compound. Carbon monoxide (CO) has a property to form complex with CYP450. The basic principle involved is mechanism based inhibition of CYP which involves following steps: 1) Preincubation of test sample with high concentration of CYP enzymes followed by dilution. 2) Incubation to measure standard enzyme activity [28].

Human/rat liver microsomes are diluted with a phospho glycerol buffer and incubated with the herbal extract under study in two different 96 well microplates. One plate is assigned as reduced P450 (P) and another is reduced P450–CO complex (PC). The reaction of herb and microsomes is initiated by addition of NADPH generating system and stopped by adding trichloro acetic acid. The PC plate is placed in the CO chamber, while the P plate is sealed with tape and placed outside the chamber. The CO chamber is designed with a plastic container and CO gas was allowed to flow into the chamber. P plate is incubated in normal

condition, while PC plate is incubated in the CO chamber for 15 min. All the samples are reduced by adding freshly prepared sodium hydrosulfite solution (SHS). At this stage, the yellow colour of the PC sample was visible whereas the P sample remained colourless or turned pale pink. Ketoconazole is an inhibitor used as positive control. The difference in absorbance of the samples at 450nm and 490nm was measured with a microplate reader. Since, reduced P450-CO complex produces a characteristic absorption peak at 450nm than reduced P450 [28,30]. Concentration of CYPs was calculated using the formula;

[CYP450] (mM) = (ΔAPC–ΔAP) / 91

Where ΔAPC = absorbance difference of the PC sample, and ΔAP = absorbance difference of the P sample, 91 is the extinction coefficient.

Percentage inhibition is determined.

Percent inhibition = (Blank – Test) / Blank × 100

Drug Transporters: P-glycoprotein (P-gp) assay

P-glycoprotein (P-gp) transporters are membrane bound proteins regulating the influx and efflux of drugs across the plasma membrane. It is found in cell membranes which affects serum drug levels by blocking or facilitating entry into cells. Also found in tissues intestine, liver and kidney. It is used widely because of simple procedure, low sample requirement, low cost, high speed and can determine IC50 value over a concentration range of >1000[31,33]. Assay is performed to determine functional activity of P-gp in cells like colon carcinoma cells (CaCo) or hepatic cells by measuring Rhodamine 123(Rh123) retention/efflux. Rh123 is a cationic, fluorescent dye taken up by the cells and actively pumped out of the cells by P-gp. Cells are thaw and viability is assessed. Cells are cultured in multiwell culture plate for 2 days. Assay is performed in triplicates. Positive control consisting of Ritonavir solution in methanol and Rh123. Control wells contain test sample, cultured cells and Rh123. Blank wells contain methanol or water, cultured cells and Rh123. Plates are incubated and after each incubation period plates are washed with ice-cold phosphate buffer saline. Fluorescence is measured using a fluorescence microplate reader. Percent inhibition calculated based on the differences in fluorescence between the test and blank wells and the mean difference between each control and blank well. IC50 values are determined. The induction of P-gp is denoted by a negative value (loss of Rh123 from the cells) and inhibition is shown by a positive value (retention of Rh123 in the cells). Percent inhibition of the test substance with respect to positive control determined [40,42].

Percentage inhibition = 100 – {(Signal of well–Blank)} × 100/ (Solvent control – Blank)

IC_{50} = {(50 – LP) × (HC – LC) + LC} / (HP – LP)

LP = Low percentage of inhibition, HP = High percentage of inhibition, LC = Low concentration, HC = High concentration

CONCLUSION

Herbals can interact with prescribed medications when taken simultaneously and can put patients at risk. The interactions often involve drug metabolizing enzymes and drug transporters. Interactions between herbal remedies and drugs lead to loss of therapeutic efficacy and toxicity. More data regarding pharmacokinetic and pharmacodynamic mechanisms of HDI is essential for clinical risk assessment. When drugs are metabolized by non-CYP enzymes it is necessary to use human hepatocytes to evaluate their ability to inhibit CYP enzymes.

Pharmacists and other health care providers must take an active role in learning about herbals and other dietary supplements to avoid herb drug interactions. Precautions should be taken as various spices and juices which we consumed in our daily life have also inhibitory potential towards these enzymes. In addition, suspected herbal drug interactions should be reported to the FDA's Adverse Event Reporting Program.

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