DRUG-NUTRIENT INTERACTIONS AND NUCLEAR RECEPTORS

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Table of Contents

1. Overview

There are several clinically relevant instances of a food or dietary supplement altering the activity of a pharmaceutical agent. For example, St. John's Wort (Hypericum perforatum), a popular herbal antidepressant, affects pharmacokinetic actions of HIV protease inhibitors, antidepressants, antihypertensives, and oral contraceptives. Drug-nutrient interaction (DNI) represents a relationship between a medication and one or more nutrients, specific food components, or metabolic status. A component of a food or dietary supplement, such as a small bioactive chemical, can affect the overall bioavailability of a drug by affecting its altered absorption, metabolism, and elimination. Small-molecule xenobiotics are metabolized in the liver and intestine by phase I and II drug-metabolizing enzymes (DMEs) and transport proteins, including influx (phase 0) and efflux (phase III) pathways. Genes involved in drug metabolism and disposition, including the predominant phase 1 enzyme system, cytochrome P450 (CYP), are transcriptionally regulated by xenobiotic-activated nuclear receptors (NRs) and transcription factors, such as PXR (pregnane X receptor), CAR (constitutive androstane receptor), FXR (farnesoid X receptor) VDR, (vitamin D receptor), AhR (aryl hyrdocarbon receptor), and Nrf2 (Nuclear factor erythroid 2-related factor 2). In general, activation of these xenobiotic receptors is considered to be predictive of a subsequent alteration in DME, which in turn impacts the bioavailability and activity of drugs and nutrients. Since many natural products and food bioactive molecules modulate activity of these xenobiotic NRs, this represents an important mechanism of DNI, namely transcription regulation of DMEs. INDIGO Biosciences has developed sensitive, specific, and robust cell-based assays for nuclear receptors, including those listed above, that are often used in high-throughput drug discovery efforts. These assays are also able to interrogate complex chemical mixtures found in botanical extracts as modulators of DME transcription.

2. Drug-Nutrient and Drug-Food Interactions

Medicinal herbs and plants have been a part of human medicine for thousands of years and continue to be an important aspect of healthcare and nutritional interventions. The World Health Organization (WHO) reported 70% of the world's population uses some form of alternative medicine, which includes herbal formulas and dietary supplements (1). Because of widespread use of both these natural products as well as pharmaceutical agents, there is increasing concern of potential drug-nutrient interactions (DNI) that may alter drugs' pharmacokinetic/ pharmacodynamic (PK/PD) profiles. This becomes especially concerning for drugs such as chemotherapeutics and immunosuppressive agents because of their narrow therapeutic index. An important mechanism by which food bioactive compounds may interfere with drug PK/PD profiles is through altering the activity of drug metabolism enzymes and transporters. Small-molecule xenobiotics are metabolized in the liver and intestine by phase I and phase II drug-metabolizing enzymes and transport proteins, including influx (phase 0) and efflux (phase III) pathways. Among the phase 1 enzyme systems, the highly polymorphic and inter-individual variable cytochrome P450 enzyme families (CYP450) are most worthy of discussion. They are an extensive family of heme-containing monooxygenases with a huge range of both endogenous and exogenous substrates. In humans, three families of CYPs mostly involved in xenobiotic metabolism are the CYP1, CYP2, and CYP3. CYP3A4 is representative among these enzymes, as it metabolizes a vast range of drugs in the liver, such as benzodiazepines, antidepressants, chemo-therapeutic agents, and calcium channel blockers (2). Among the herbs, St. John's Wort (Hypericum perforatum), a popular herbal antidepressant, has become the most studied natural product in terms of drug-nutrient interaction. St. John's Wort has been demonstrated to have clinically relevant pharmacokinetic effects, leading to decreased plasma bioavailability of HIV protease inhibitors, antidepressants, antihypertensives, cardiovascular medicines, blood pressure medicines, bronchodilators, immune-suppressants, sedatives, and steroid hormones such as oral contraceptives (3-5). As reviewed in Briguglio et al. (2), several foods and bioactive molecules affect pharmacodynamic processes, perhaps through interacting with receptors, including: ginger, camellia tea, poppy seed, bean sprout, strawberry, tomato, swede, fennel, celery, licorice, guava, sugar-pea, mango, maize, turnip, rice, and avocado.

3. Regulation of Drug-Metabolism Enzymes by Xenobiotic Nuclear Receptors and Transcription Factors

As mentioned above, changes in DME and transporter activity can result in altered exposure and tissue distribution of drugs and may manifest as adverse effects or therapeutic failure. The coordinate regulation of drug metabolism and excretion is due largely to the activity of ligand-activated nuclear receptors and transcription factors (see Figure 1). Specifically, PXR (pregnane X receptor), CAR (constitutive androstane receptor), FXR (farnesoid X receptor), VDR (vitamin D receptor), AhR (aryl hydrocarbon receptor, and Nrf2 (Nuclear factor erythroid 2-related factor 2) are responsible for the transcription regulation of a variety of clinically relevant DMEs and transporters (6-8). Of the NRs listed above, PXR (NR1I2) represents perhaps the most important and well-studied in terms of DNI. This promiscuous receptor is activated by a structurally diverse collection of chemicals, including both xenobiotics and endogenous chemicals.

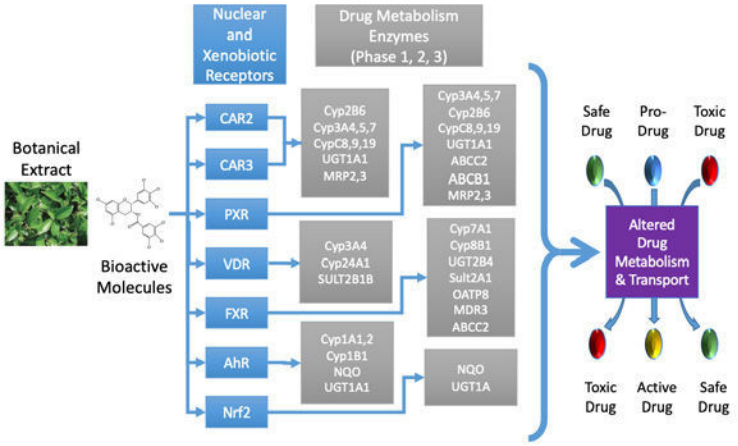

Figure 1. Adverse Outcome Pathways (AOP) for Drug-Nutrient Interaction (DNI) via Transcription Regulation of Drug Metabolism

Activation of PXR is associated with the induction of many target genes including CYP3A4, UGT1A1, and multidrug resistance protein MDR1 P-glycoprotein (Pgp) (9, 10). Since CYP3A4 metabolizes approximately 50% of all prescription drugs, this represents an important mechanism of DNI, as demonstrated by the fact that hyperforin found in St. John's Wort is responsible for altered PD/PK properties of a wide range of drugs through acting as a PXR ligand. The human constitutive androstane receptor (CAR, NR1I3) regulates the expression of genes involved in xenobiotic metabolism and transport in the liver, including CYP2B and 3A4, UGT1, and MDR1 (9).

Interestingly, this receptor has several splice variants in humans (CAR1, 2, and 3), each with different ligand preferences (9). The VDR (NR1I1) is the endogenous receptor for the active form of vitamin D, 1,25(OH)2D3 and, like its counterparts in the NR family 1I (PXR and CAR), regulates the expression of CYP3A4 and UGT1A1. The main physiological role of FXR (NR1H4) is to act as a bile acid sensor in the enterohepatic tissues (10). FXR activation regulates the expression of various transport proteins and DMEs crucial to the physiological maintenance of bile acids and lipid and carbohydrate metabolism; there are several phytochemicals and dietary fatty acids that act as FXR ligands (11; 12). AhR is a member of the basic-helix-loop-helix-Per-Arnt-Sim (bHLH-PAS) gene superfamily of transcription factors (13). AhR is known to recognize a range of chemical structures, including non-aromatic and non-halogenated compounds with the prototypical agonist being 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), and regulate DMEs, in particular CYP1A1 and 1A2. A number of reports have identified specific dietary constituents that are AHR ligands such as the flavonoids quercetin, apigenin, and kaempferol and components of cruciferous vegetables, such as indole-3-carbinol (14). Nuclear erythroid 2-related factor 2 (Nrf2) is a transcription factor that belongs to the Cap-n-collar basic leucine zipper family (15). Nrf2 is considered the main mediator of cellular adaptation to redox stress with heme oxygenase-1 (HO-1), NAD(P)H: quinone oxidoreductase 1 (NQO1), glutathione-S-transferases (GST), group C streptococcus (GCS) and is involved in the detoxification of increased electrophiles and radicals. The important grape skin bioactive, resveratrol, is a known activator of Nrf2 and may be responsible for some of its biological effects (8).

4. Example Data

a. Regulation of Xenobiotic Nuclear and Transcription Factors by Dietary Supplements

We have used cell-based reporter assays and gene expression as tools to understand the biological activity of botanical extracts, food products, and natural bioactive molecules, including walnuts (11; 12; 16), pistachio (17), cranberry (18), spice mixes (19; 20), and a variety of dietary fatty acids (21-25). Based on the experience described above, we have designed a panel of xenobiotic nuclear receptors and transcription factors (DNI Panel, human AhR, CAR2, CAR3, FXR, Nrf2, PXR, and VDR), that can be used to examine the AOP shown in Figure 1. Several botanical extracts at their maximum tolerated concentration were examined in the DNI panel (left side of Figure 2) with Ashwaganda, oak straw, and bacopa significantly increasing activity. Subsequently, when individual receptors were examined, all three positive extracts activated AhR, while oak straw and bacopa increased CAR2 activity (right side of Figure 2).

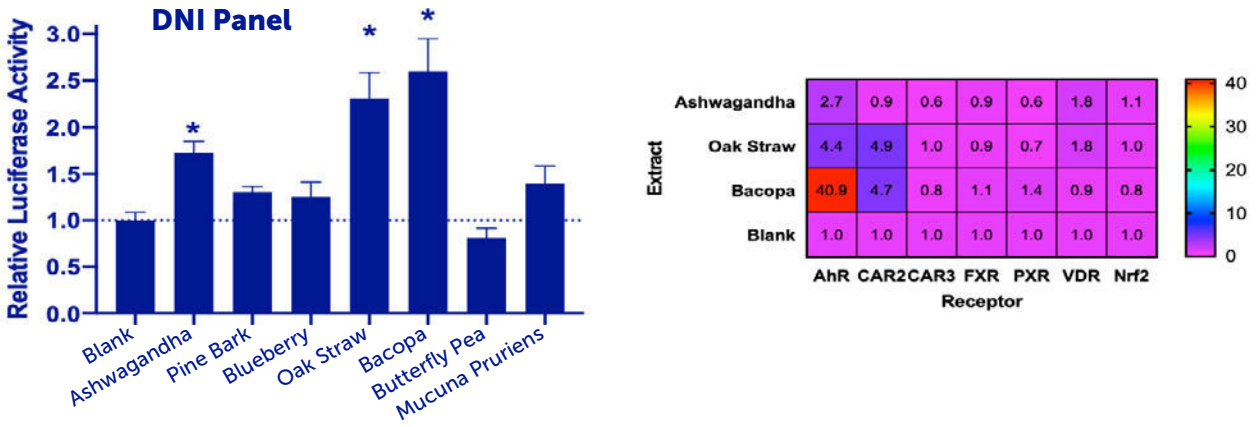

Figure 2. Use of the DNI AOP Approach (Tier 1 and Tier 2) for Selected Botanical Extracts

b. DME Regulation in upcyte® Human Hepatocytes

upcyte® hepatocytes were treated with prototypical ligands of the xenobiotic-sensing nuclear receptors PXR, CAR, AhR, FXR, and PPAR. Treatment concentrations were determined to be non-toxic to the hepatocytes. Following 48-hour treatment, cell lysates were prepared and total RNAs were purified. Quantitative PCR (qPCR) was performed using SYBR green reaction chemistry and validated primer sets for CYP3A4, CYP1A1, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2E1, and ACTB. Drug-induced changes in the expression of the CYPs were quantified using the ΔΔCt analytical method; the expression of ACTB was used as the internal reference for normalizing qPCR results between sample replicates. Depicted in Figure 3 are fold-changes in CYP gene expression relative to DMSO (0.1% v/v) treated controls. Rifampicin, ß-naphthoflavone, and CDCA are inducers of PXR, AhR, and FXR respectively and regulate the CYPs depicted in Figure 1. Therefore, upcyte® human hepatocytes are an effective model system for examining altered DME by botanical extracts.

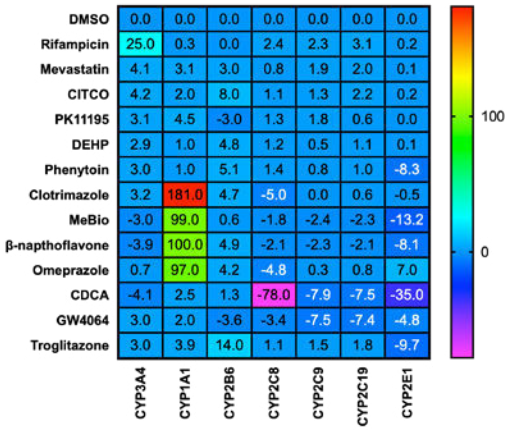

Figure 3. Regulation of DMEs by Reference Compounds in upcyte® Hepatocytes

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