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Research Paper

Interactions of Papua New Guinea medicinal plant extracts with antiretroviral therapy

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ABSTRACT

Ethnopharmacological relevance: A substantial proportion of the population in Papua New Guinea (PNG) lives with human immunodeficiency virus (HIV). Treatment requires lifelong use of antiretroviral therapy (ART). The majority of people in PNG use traditional medicines (TM) derived from plants for all types of health promotions. Consequently, there is a concern that herb–drug interactions may impact the efficacy of ART. Herb–drug, or drug–drug, interactions occur at the level of metabolism through two major mechanisms: enzyme induction or enzyme inhibition. In this study, extracts of commonly-used medicinal plants from PNG were screened for herb–drug interactions related to cytochrome P450s (CYPs).

Materials and methods: Sixty nine methanol extracts of TM plants were screened for their ability to induce CYPs by human aryl hydrocarbon receptor- (hAhR-) and human pregnane X receptor- (hPXR-) dependent mechanisms, utilizing a commercially available cell-based luciferase reporter system. Inhibition of three major CYPs, CYP1A2, CYP3A4, and CYP2D6, was determined using human liver microsomes and enzyme-selective model substrates.

Results: Almost one third of the TM plant extracts induced the hAhR-dependent expression of CYP1A2, the hPXR-dependent expression of CYP3A4, or both. Almost two thirds inhibited CYP1A2, CYP3A4, or CYP2D6, or combinations thereof. Many plant extracts exhibited both induction and inhibition properties.

Conclusions: We demonstrated that the potent and selective ability of extracts from PNG medicinal plants to affect drug metabolizing enzymes through induction and/or inhibition is a common phenomenon. Use of traditional medicines concomitantly with ART could dramatically alter the concentrations of antiretroviral drugs in the body; and their efficacy. PNG healthcare providers should counsel HIV patients because of this consequence.

1. Introduction

Papua New Guinea (PNG) is in the midst of an HIV epidemic. According to the UNAIDS, 2013 *Report on the Global AIDS Epidemic*, an estimated 25,000 people in PNG are living with HIV (approximately 1% of the population) (UNAIDS, 2013). Standard treatment of HIV patients requires a lifelong regimen of antiretroviral therapy (ART). PNG is culturally diverse (Whitehead, 1994) and traditional medicines play a vital role in the well-being of the people (National Department of Health, 2007). Many people, especially in regions where access to Western medicine is limited, rely on medicinal plants to relieve an array of ailments (Rai, 2007). Medicinal herb–drug interactions have been studied in areas that rely heavily of traditional medicines around the world (Müller and Kanfer, 2011; Lau et al., 2013). However, little is known about herb–drug interactions that may arise from PNG medicinal plants in combination with ART.

Drug–drug interactions involving cytochrome P450s (CYPs) occur through two major mechanisms: enhanced enzyme expression, termed induction, and enzyme inhibition. With the exception CYP2D6, the CYPs responsible for drug metabolism in the liver are

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inducible. CYP induction and the resultant increased drug metabolism leads to a decrease in bioavailable drug and a decrease in drug efficacy. Inhibition of CYPs, on the other hand, decreases drug metabolism and results in an increase in bioavailability. Elevated levels of drugs may enhance their therapeutic benefits, but can result in toxicity.

Particularly worrisome is the possibility that traditional medicine usage may unknowingly render normal ART ineffective. We surveyed 69 methanol extracts of commonly-used medicinal plants from 7 PNG provinces for CYP induction and inhibition. Induction profiles were determined for CYP1A2 and CYP3A4, enzymes whose expression levels are elevated by two distinct pathways. The inhibition profiles were determined for CYP1A2, CYP3A4, and CYP2D6, which are estimated to be responsible for biotransformation of approximately two thirds of prescription drugs (Wrighton and Stevens, 1992). Almost one third of the medicinal plant extracts tested significantly induced CYP expression, while close to two thirds exhibited potent CYP inhibition.

2. Materials and methods

2.1. Medicinal plant collection

As previously reported (Waruruai et al., 2011; Jorim et al., 2012), University of Papua New Guinea (UPNG) fourth year pharmacy students collected medicinal plants from their home communities. These were in the Eastern Highlands, Western Highlands, Southern Highlands, Enga, Western, Manus and Northern Bougainville (autonomous) provinces. These surveys served to fulfill requirements for a Bachelor of Pharmacy degree from UPNG. In accordance with the requirements, plant information, including local names and medicinal uses, was documented. Voucher samples were prepared for plant identification and the information was compiled into a formal report. Plants were identified by UPNG or National herbaria staff where vouchers are stored. The reported data are stored in the UPNG Traditional Medicines Database. A compilation of this information is presented in **Appendix 1**.

2.2. Medicinal plant extraction

Dried, plant samples (~10 g) were extracted in 100 mL of 100% methanol (MeOH) overnight. Extracts were then evaporated to dryness and dissolved in dimethyl sulfoxide (DMSO) to yield a final concentration of approximately 10 mg/mL. Fractionation of *Evodia hortensis* was accomplished by mixing MeOH extract with three times its dry weight of Diaion® HP20SS resin (Sorbent Technologies). The extract and resin mixture was evaporated to dryness, loaded into a column, and extracted using stepwise increases of isopropanol (IPA). The fractions were eluted as follows: 10% IPA/H_2O (FW), 25% IPA/H_2O (F1), 50% IPA/H_2O (F2), 75% IPA/H_2O (F3), and 100% MeOH (F4) (Bugni et al., 2008). These were evaporated to dryness and dissolved in DMSO to a final concentration of approximately 10 mg/mL.

2.3. CYP induction assays

Plant extracts were screened for the ability to induce CYP1A2 and CYP3A4. 1A2DRE (human XRECYP1A2-luciferase promoter) and DPX2 (human PXRCYP3A4-luciferase promoter) hepatoma cell lines (Puracyp, Inc.) were grown in T75 flasks to near-confluency (~75%). The cells were trypsin-released and aliquoted into 96-well plates. Cells were allowed to adhere for 24 h. Plant extracts were added to the wells and incubated for another 24 h. Cell viability and luciferase activity were then determined using commercially-available kits

Table 1
CYP1A2 and CYP3A4 inducers and their inhibitory activities.

Genus and species	Voucher number	Plant part	PNG province	CYP1A2 Induction (-fold)	CYP3A4 Induction (-fold)	Inhibits CYP1A2	Inhibits CYP3A4	Inhibits CYP2D6
P. conjugatum	cw070	spikelets	Western Highlands	**11.4**	—	✓	✓	✓
S. rhombifolia	nn010	leaves	Manus	**15.5**	—	✓		
A. scholaris	in012	leaves	North Bougainville	—	**4.7**			✓
B. pilosa	em028	leaves	Southern Highlands	—	**5.7**			
B. pilosa	cw004	leaves	Western Highlands	—	**5.5**			✓
C. blumei	sk043	whole plant	Eastern Highlands	—	**4.0**			✓
C. terminalis	cw030	leaves from shoots	Western Highlands	—	**3.8**		✓	✓
C. longa	jp1103	leaves	Enga	—	**5.8**			
M. esculenta	cw023	leaves	Western Highlands	—	**3.7**		✓	
P. pinnata	ab057	bark	Western	—	**4.2**	✓	✓	✓
P. guajava	sk002	leaves	Eastern Highlands	—	**5.2**	✓	✓	✓
P. guajava	nn011	leaves	Manus	—	**4.6**	✓	✓	
P. guajava	in045	leaves	North Bougainville	—	**8.7**		✓	✓
P. guajava	em009	leaves	Southern Highlands	—	**5.8**	✓	✓	✓
P. guajava	cw019	leaves	Western Highlands	—	**3.5**	✓	✓	
W. biflora	in021	leaves	North Bougainville	—	**3.8**			
C. inophyllum	nn021	leaves	Manus	**31.7**	**15.6**	✓	✓	✓
C. variegatum	nn016	leaves	Manus	**5.8**	**14.2**		✓	✓
E. variegata	nn056	bark	Manus	**4.5**	**3.6**	✓	✓	✓
E. hortensis	cw049	leaves & fruits	Western Highlands	**13.7**	**7.1**	✓	✓	✓
S. unitus	in061	shoots	North Bougainville	**14.9**	**5.0**	✓	✓	

Medicinal plant induction of CYP1A2 and/or CYP3A4. (—) activity was < 4.0-fold (CYP1A2) & < 3.5-fold (CYP3A4); (✓) indicates inducers that also inhibited CYP metabolism.