INHIBITION OF HUMAN CYTOCHROME P450 ACTIVITIES BY KAVA EXTRACT AND KAVALACTONES

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ABSTRACT:
The herb kava has recently been associated with numerous drug interactions, but its interaction with cytochrome P450 (P450) enzymes has not been investigated. In the present work the inhibition of P450 enzymes by kava extract and individual kavalactones in human liver microsomes (HLMs) was investigated. Whole kava extract (normalized to 100 μM total kavalactones) caused concentration-dependent decreases in P450 activities, with significant inhibition of the activities of CYP1A2 (56% inhibition), 2C9 (92%), 2C19 (86%), 2D6 (73%), 3A4 (78%), and 4A9/11 (65%) following preincubation for 15 min with HLMs and NADPH; CYP2A6, 2C8, and 2E1 activities were unaffected. The activities of CYP2C9, 2C19, 2D6, and 3A4 were also measured after incubation of HLMs with the major kavalactones kawain (K), desmethoxyyangonin (DMY), methysticin (M), dihydromethysticin (DHM) (each at 10 μM), and NADPH. Whereas K did not inhibit these enzymes, there was significant inhibition of CYP2C9 by DMY (42%), M (58%), and DHM (69%); of 2C19 by DHM (76%); of 2D6 by M (44%); and of 3A4 by DMY (40%), M (27%), and DHM (54%). Consistent with their potency as inhibitors, the two major kavalactones bearing a methylenedioxyphenyl moiety (M and DHM) formed “455 nm” metabolic intermediate complexes after incubation with HLMs and NADPH, but K and DMY did not. These data indicate that kava has a high potential for causing drug interactions through inhibition of P450 enzymes responsible for the majority of the metabolism of pharmaceutical agents.

Kava, Piper methysticum, is a shrub indigenous to the islands of the South Pacific (Rasmussen et al., 1979; Rouse, 1998). Inhabitants of this region prepare an aqueous extract of the root for use as a ritual beverage known for promoting relaxation and a sense of well being (Keledjian et al., 1988). The pharmacology, toxicology, and chemical characterization of kava have been reviewed extensively (Lebot et al., 1992; Jellin et al., 2002). Fifteen lactones, termed kavalactones, have been identified in kava root extract. Yangonin, desmethoxyyangonin (DMY)1; 5,6-dehydrokawain, methysticin (M), 7,8-dihydromethysticin (DHM), kawain (K), and 7,8-dihydromethysticin (Fig. 1) are present in the highest concentrations and account for approximately 96% of lipidic extracts (Lebot and Lévesque, 1989).

Case reports detailing liver failure after ingestion of kava have been emerging over the last few years (Strahl et al., 1998; Kraft et al., 2001), and over-the-counter sales of these herbal preparations have recently been banned in Switzerland and Germany; Britain is also proposing a ban (Jellin et al., 2002). Heavy kava consumption has been linked to a characteristic dermatopathy, termed kani, consisting of rough and scaly skin with light and dark bands (Keledjian et al., 1988) that is possibly attributable to altered cholesterol metabolism (Ruze, 1990; Lebot et al., 1992). There are reports of significant drug interactions when kava is used concomitantly with alprazolam (Almeida et al., 1996) and other central nervous system depressants, including barbiturates, benzodiazepines, and alcohol (Blumenthal et al., 1998; DerMarderosian, 1999).

When administered individually, kavalactones do not exhibit the degree of pharmacological activity observed after administration of whole kava extract (Lebot et al., 1992), possibly indicating modulation of transport or metabolism. Although no detailed reports of the inhibition of human cytochrome P450 (P450) enzymes by kava constituents were found in the literature, Meyer (1962) demonstrated a dramatic increase in hexobarbital sleep time, possibly indicative of CYP2C inhibition, following administration of kavalactones to mice. Additionally, methysticin analogs present in kava extract contain a methylenedioxyphenyl group. This moiety has been shown, after metabolic activation, to inhibit multiple cytochrome P450 enzymes through the formation of metabolic intermediate (MI) complexes (Hodgson and Philpot, 1974; Murray et al., 1983; Murray and Reidy, 1989). The present studies were conducted to help elucidate how the constituents of kava may interact synergistically, and to help explain and predict interactions with drugs with which kava is taken concomitantly.

Materials and Methods

Chemicals. KavaPure Kava PE 40%, powdered kava extract, was obtained from PureWorld Botanicals, Inc. (South Hackensack, NJ). Individual kavalactones, including desmethoxyyangonin (5,6-dehydrokawain), 7,8-dihydromethysticin, kawain, methysticin, and yangonin, were isolated from kava and supplied by ChromaDex, Inc. (Laguna Hills, CA). All other chemicals and reagents used were of the highest commercially available quality.

Human Liver Microsomes (HLMs). A pooled sample of human liver...
Human liver microsomes were incubated with kava extract or individual kavalactones and NADPH prior to determination of individual P450 enzyme activities. Enzymes and their activities are as follows: CYP1A2, acetanilide hydroxylase; CYP2A6, coumarin 7-hydroxylase; CYP2C8, paclitaxel 6α-hydroxylase; CYP2C9, tolbutamide hydroxylase; CYP2C19, mephenytoin 4-hydroxylase; CYP2D6, dextromethorphan O-demethylase; CYP2E1, p-nitrophenol hydroxylase; CYP3A4, dextromethorphan N-demethylase; CYP4A9/11, lauric acid α-hydroxylase. Significantly different from control, *, p < 0.05; **, p < 0.01; ***, p < 0.001.

**Fig. 1.** Activities of hepatic microsomal cytochrome P450 enzymes after incubation of human hepatic microsomes with kava extract and individual kavalactones. Human liver microsomes were incubated with kava extract or individual kavalactones and NADPH prior to determination of P450 enzymatic activities. Enzymes and their activities are as follows: CYP1A2, acetanilide hydroxylase; CYP2A6, coumarin 7-hydroxylase; CYP2C8, paclitaxel 6α-hydroxylase; CYP2C9, tolbutamide hydroxylase; CYP2C19, mephenytoin 4-hydroxylase; CYP2D6, dextromethorphan O-demethylase; CYP2E1, p-nitrophenol hydroxylase; CYP3A4, dextromethorphan N-demethylase; CYP4A9/11, lauric acid α-hydroxylase. Significantly different from control, *, p < 0.05; **, p < 0.01; ***, p < 0.001.
In subsequent experiments, those P450 enzymes that were most susceptible to inhibition by kava extract were assayed in the presence of individual kavalactones. HLMs were preincubated for 15 min with NADPH and 1 or 10 μM K, DMY, M, or DHM prior to the determination of CYP2C9, 2C19, 2D6, and 3A4 activities. Whereas K did not inhibit these enzymes, there was significant inhibition of CYP2C9 by DMY (42%); M (58%); and DHM (69%); of 2C19 by DHM (76%); of 2D6 by M (44%); and of 3A4 by DMY (40%), M (27%), and DHM (54%) when incubated at a kavaloactone concentration of 10 μM.

In experiments designed to determine whether kava constituents form MI complexes with P450 enzymes, human hepatic microsomes were incubated in the presence of whole kava extract or individual kavalactones, and the P450 difference spectra were measured from 400 to 480 nm (Fig. 2). Microsomes incubated in the absence of any kava constituent exhibited a difference spectrum with a single peak at the absorbance maximum of 427 nm after addition of NADPH. Similar results were obtained with the kava constituents DMY and K. However, incubation of microsomes in the presence of kava extract or its major methylenedioxyphenyl-containing constituents, M and DHM, resulted in the production of a new absorption with a maximum at 455 nm. Production of the 455-nm absorbing complex was both NADPH- and time-dependent, with absorption increasing with increasing incubation time. The classic MI complex-forming agent, piperonyl butoxide (1 mM), produced the same magnitude of 455-nm complex as did DHM and M (data not shown).

**Discussion**

The herb kava has attracted considerable recent attention due to regulatory scrutiny in Europe and the United States. These actions were precipitated by reports of liver failure and reports that the "pattern of (P450) enzymes shifts after ingestion of kava kava" (American Botanical Council, 2001). In the present work, the interaction of human P450 enzymes with kava extract and its constituents was investigated in human liver microsomes. CYP1A2, 2C9, 2C19, 2D6, and 3A4 were all markedly inhibited at a kavaloactone concentration of 100 μM; this is the first literature report of the effect of kava and its constituents on P450 enzymes. A number of accounts have provided indirect indications that kava inhibits P450 in vivo, but they have not been interpreted as such. The data herein give pause for a case-by-case reevaluation of the causes of these adverse effects.

In addition to liver failure, and terse "letter to the editor" reports linking hepatotoxicity to low CYP2D6 activity, there are similar reports linking lethargy and coma to concomitant use of benzodiazepines and other central nervous system depressants with kava. The prevailing hypothesis for the latter is that this is due to exaggeration of pharmacological response, consistent with the GABA agonist activity of kavalactones (Jussofie et al., 1994). Based on their observations of poor debrisoquine metabolism in two individuals reporting symptoms of liver damage following kava ingestion, Russmann et al. (2001) postulated that CYP2D6 deficiency is a risk factor for kava hepatotoxicity. However, based on the findings of the current study and the low prevalence of CYP2D6 deficiency in the general population, it would appear that inhibition of CYP2D6 expressed at normal levels, rather than low genotypic expression, alone, could account for the poor debrisoquine metabolism observed in these patients who had consumed kava.

Case reports and pharmacologic studies have indicated a potentiality of the central nervous system effects of benzodiazepines, including alprazolam, in the presence of kava extract and/or kavalactones (Jussofie et al., 1994; Almeida and Grimsley, 1996). Alprazolam clearance in humans correlates with CYP3A4/5 activity (Schmider et
al., 1999; D’Souza et al., 2001; Hirota et al., 2001), as does the clearance of other benzodiazepines. Inhibition of benzodiazepine metabolism as a result of decreased CYP3A4 activity may contribute to the additive effects reported upon coadministration of kava and alprazolam. At the outset of these investigations, one of the few reports immediately suggesting inhibition of P450 arose from the observation that coadministration of kava markedly increased hexobarbital sleep time in mice, indicative of inhibition of CYP2C (Meyer, 1962). CYP2C9 metabolizes hexobarbital in humans (Guengerich, 1995), and the extensive inhibition of this family shown herein is consistent
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with the effects found in mice. CYP2C9 is also important in the metabolism of other barbiturates, nonsteroidal anti-inflammatory drugs, and the anticoagulant warfarin. The risk of severe bleeding due to concomitant ingestion of kava with warfarin, which has a narrow therapeutic index, is but one example of the potential consequences of the inhibition of this and other P450 enzymes.

By 1984, in Vanuatu alone, 72 different cultivars of kava had been identified (Ellis, 1984). Because these cultivars vary greatly in chemical composition and physiological effects, Lebot and Levesque (1989) characterized them based on their relative concentrations of six kavalactones: desmethoxyyangonin, 7,8-dihydrokawain, yangonin, kawain, dihydromethysticin, and methysticin. Among the cultivars characterized was a variety known in the vernacular of Vanuatu as ‘‘tudei’’ (‘‘two days’’), renowned for its intense physiological effects and an intoxication that lasts for 2 days. This cultivar was found to contain particularly high concentrations of methysticin and dihydromethysticin, kavalactones that contain a methylenedioxyphenyl functional group. In the present studies, incubation of HLMs with either M or DHM resulted in the time- and NADPH-dependent formation of an MI complex with a characteristic absorbance maximum at 455 nm, indicating the inactivation of P450 enzymes. However, the magnitude of the 455-nm absorbance was not pronounced. Since DHM, as well as DHM and M, caused the most pronounced enzyme inhibition, it may be that competitive inhibition is a major contributor to the overall inhibition of P450 activities. Alternatively, displacement of MI complexes by the presence of high concentrations of unmetabolized kavalactone (initial concentration, 1 mM), as has been shown for other lipophilic displacers (Elcombe et al., 1975), may have diminished the amount of complex ultimately measured.

The profound, long-term, and broad spectrum inhibition of human P450 enzymes responsible for the metabolism of >90% of pharmaceuticals poses a significant risk for consumers. The authors suggest that fractionation or commercial development of cultivars containing lesser amounts of methysticin and 7,8-dihydromethysticin, and higher kavalactone content, could alleviate drug interactions associated with this herb, while preserving many of its beneficial properties.

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Rasmussen AK, Scheline RR, Solheim E, and Hansel R (1979) Metabolism of some kava lactones (initial concentration, 1 mM), as has been shown for other lipophilic displacers (Elcombe et al., 1975), may have diminished the amount of complex ultimately measured.

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