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Effects of herbal products and their constituents on human cytochrome $P450_{2E1}$ activity

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Abstract

Ethanolic extracts from fresh *Echinacea purpurea* and *Spilanthes acmella* and dried *Hydrastis canadensis* were examined with regard to their ability to inhibit cytochrome $P450_{2E1}$ mediated oxidation of *p*-nitrophenol in vitro. In addition, individual constituents of these extracts, including alkylamides from *E. purpurea* and *S. acmella*, caffeic acid derivatives from *E. purpurea*, and several of the major alkaloids from *H. canadensis*, were tested for inhibition using the same assay. *H. canadensis* (goldenseal) was a strong inhibitor of the $P450_{2E1}$, and the inhibition appeared to be related to the presence of the alkaloids berberine, hydrastine and canadine in the extract. These compounds inhibited 2E1 with K_I values ranging from 2.8 μM for hydrastine to 18 μM for berberine. The alkylamides present in *E. purpurea* and *S. acmella* also showed significant inhibition at concentrations as low as 25 μM, whereas the caffeic acid derivatives had no effect. Commercial green tea preparations, along with four of the individual tea catechins, were also examined and were found to have no effect on the activity of $P450_{2E1}$.

Keywords: Echinacea; Cytochrome $P450_{2E1}$; Hydrastis; Goldenseal; Spilanthes; Green tea

1. Introduction

Cytochrome $P450_{2E1}$ is an alcohol inducible P450 isoform that has been implicated in the generation of reactive oxygen species such as superoxide and hydrogen peroxide and may mediate the toxic effects of a variety of xenobiotic compounds (Gorsky et al.,1984; Guengerich et al., 1991; Castillo et al., 1992). This isoform has been identified in a wide range of tissue types outside the liver, including, intestines (Subramanian and Ahmed, 1995), tongue (Yang et al., 2003), kidney, and nasal mucosa (Ding et al., 1986). The widespread distribution of this isoform and its potential role in activating toxins, along with the observed induction by exposure to alcohol, suggest that compounds with the ability to inhibit $P450_{2E1}$ may be useful in the prevention of deleterious effects of various chemical toxins that are activated by this enzyme. For example, Xu et al. (2003) have shown that exposure of human hepatoma cells (HepG2) to arachidonic acid results in the generation of hydrogen peroxide, and that cells overexpressing cytochrome $P450_{2E1}$ produce elevated levels of hydrogen peroxide, implicating this P450 isoform in its formation. The hydrogen peroxide produced in these cells is genotoxic due to resulting lipid peroxidation. Moreover, treatment of the cells with the retinoid lycopene protected the cells from the $P450_{2E1}$-derived peroxide. Others have also demonstrated that inhibition of cytochrome $P450_{2E1}$ can lead to reduction in the genotoxicity of other xenobiotics whose toxicity is mediated by this enzyme (Hammond and Fry, 1997).

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Herbal extracts hold promise for use in strategies for inhibiting the metabolic activation of toxic xenobiotics. Such extracts contain a diverse array of chemical species, each with the potential to act on specific cytochrome P450 enzymes in an inhibitory manner. Many examples of herbs or food products that interact with cytochrome P450 enzymes exist in the literature (Brady et al., 1988; Guengerich and Kim, 1990; Subehan et al., 2006; Koul et al., 2000), and in some instances, drug interactions have been observed (Bailey et al., 1998; Wentworth et al., 2000). However, the complex nature of the extracts is a problem from a regulatory standpoint and in understanding the biochemical affects they exert on xenobiotic metabolizing systems. Thus, it is important not only to recognize the inhibitory effect of a complete extract, but to also identify the individual components of the extract that contribute to the observed inhibitory effect.

The herbal products *Echinacea purpurea*, *Hydrastis canadensis*, green tea, and *Spilanthes acmella* are popular products that are widely used for medicinal purposes. The effects of some of these products on drug metabolism in various tissue types have been studied (Budzinski et al., 2000; Yale and Glurich, 2005; Yang and Raner, 2005; Gorski et al., 2004; Chatterjee and Franklin, 2003). However, the ethanol inducible cytochrome $P450_{2E1}$ enzyme is often neglected in such pharmacologically driven studies. Consequently, little is known regarding the potential interaction of cytochrome $P450_{2E1}$ with these natural products. Given the possible role of $P450_{2E1}$ in the generation of reactive oxygen species in the liver, and the resulting oxidative stress associated with these species, interactions that could reduce $P450_{2E1}$ activity under certain conditions may prove beneficial.

In the current study, we have examined preparations of four different medicinal plants, cone flower (*E. purpurea*), toothache plant (*S. acmella*), goldenseal (*H. canadensis*) and green tea (*Camellia sinensis*). Their abilities to inhibit cytochrome $P450_{2E1}$ from human liver microsomes and from an in vitro expression system have been evaluated. In addition, we have selected several major classes of chemical species found in each of the extracts and tested the individual compounds with respect to their inhibitory properties on the human $P450_{2E1}$ isoform.

2. Materials and methods

2.1. Chemicals and enzymes

All of the chemicals used in this study except for the alkylamides from *E. purpurea* and *S. acmella* were purchased from commercial suppliers Sigma Aldrich or Acros Chemical Companies. *E. purpurea* fresh roots were purchased from Pacific Botanicals (Williams, OR) and *S. acmella* fresh plants were purchased from Horizon Herbs (Williams, OR). Ethanolic extracts (95% and 33%) were prepared from this plant material according to published procedures (Cech et al., 2006a). Dried roots of *H. canadensis* were also obtained from Horizon Herbs and were extracted at a ratio of 1:5 (1 mL solvent:5 g powdered roots) in 50% ethanol:50% water. A commercial green tea extract (standardized to contain 50% polyphenols) manufactured by Spring Valley (Bohemia, NY) was used.

2.2. Purification of alkylamides from S. acmella and E. purpurea

Alkylamides were separated from the 95% ethanol extracts of *S. acmella* and *E. purpurea*. Concentrated extract (100 mL) was diluted to 200 mL with de-ionized water. A 35 g C18 reverse phase extraction cartridge was equilibrated with 100 mL of a 50% ethanol:50% H_2O solution. The diluted *E. purpurea* solution was loaded onto the cartridge at a flow rate of 7 mL/min. After the entire 200 mL had passed through, the cartridge was washed with 100 mL of 50% ethanol:50% H_2O. The eluent was collected in 25 mL fractions and stored for later analysis. Three subsequent elutions were carried out in the same manner. The mobile phase for these three elutions consisted of 55% ethanol:45% H_2O, 60% ethanol:40% H_2O and 70% ethanol:30% H_2O, respectively. A total of four-25 mL fractions were collected from each of the washing steps for a total of 16 fractions. At this point, each of the fractions was analyzed by HPLC to identify those containing alkylamides. Fractions containing significant amounts of the alkylamides were then submitted to HPLC purification using a semi-preparative C18 column (250×10 mm) with an injection volume of up to 5 mL. The column was first equilibrated in 50% acetonitrile containing 0.1% trifluoroacetic acid and the sample was analyzed using the same mobile phase with a flow rate of 1.5 mL/min and a detection wavelength of 200 nm. Individual peaks from the chromatogram were collected and the mobile phase was evaporated. Identity of the alkylamides in the fractions was verified using an LC/MS assay described previously (Cech et al., 2006a). An identical procedure was used to purify several *S. acmella* alkylamides.

2.3. Quantification of alkylamides in E. purpurea and S. acmella extracts

Since standards for each of the alkylamides are not commercially available, a single compound (dodeca-2E,4E,8Z,10Z-tetraenoic acid isobutlyamide) was purchased from Chromadex (Santa Anna, CA) and used as a standard for quantification of all of the isobutylamides isolated. It was assumed that the spectrophotometric properties of all of the alkylamides would be similar enough for this approach to provide a reasonable estimate of the concentrations of each in the complex extracts.

2.4. Cytochrome $P450_{2E1}$ assay

Cytochrome $P450_{2E1}$ activity was monitored using the substrate *p*-nitrophenol in human liver microsomes and expressed cytochrome $P450_{2E1}$. The $P450_{2E1}$ assay used in this laboratory has been reported previously, and was used without modification for control reactions (Larson et al., 1991). The inhibitory properties of the extracts and individual components were determined by including a known quantity of the inhibitor in the $P450_{2E1}$ assay and monitoring the effects on V_{max} and K_m for the reaction. In all cases, if ethanol or methanol was present in the stock solution of inhibitor, the solvent was evaporated, and the residue was re-dissolved in the assay buffer prior to adding the enzyme and remaining components, since ethanol is a known inhibitor of $P450_{2E1}$. This also ensured that the inhibitor was soluble in the reaction mixture at the concentrations used.

2.5. Quantification of individual components in various extracts

The four major catechins in green tea extract, EGCG, EC, EGC and ECG were quantified in a sample of the extract using a three point calibration curve as described previously (Lee et al., 2000). Caffeic acid derivatives, caftaric acid, chlorogenic acid and cichoric acid were analyzed in the 33% ethanolic *E. purpurea* extract using HPLC separation followed by ESI-MS for detection and quantification (Sasagawa et al., 2006). The HPLC system used was an HP1100 by Agilent with a C18 (50×2.1, 3.0 μm particle size) column. Injection volume was 10 μL and flow rate set at 0.2 mL/min. Calibration curves were generated using a mixture of standard compounds purchased from Chromadex, Inc. (Santa Anna, CA, USA). The alkaloids berberine, hydrastine and canadine were quantified