Drug-herb interaction potential: effects on cytochrome P450 and P-glycoprotein

In this guide

In vitro and animal studies

60. The in vitro inhibition potential of E. purpurea against baculovirus-expressed cytochrome P450 (CYP) 3A4, 2D6 and 2C9 enzymes was evaluated (Yale and Glurich, 2005). One capsule of EchinaCare containing 50 mg of 50:1 E. purpurea aerial parts extract was sonicated for 5 minutes in 70% methanol, the supernatant was collected and diluted 1:10. This was designated as 100% extract and used in the in vitro assay. High throughput screening was performed by incubating 1:3 serial dilutions of the 100% extract with the enzymes and

substrate for 45 minutes prior to measuring the metabolite fluorescence. The assays were run in duplicate, and the 50% inhibitory concentration (IC50) was determined from the fluorescence values, signifying the percentage inhibition, against the extract concentration plots. The Echinacea extract showed virtually no inhibition against CYP2D6. There was a mild inhibition of CYP3A4 with IC50 equal to 75% extract. Maximum inhibition of CYP2C9 was seen at 33% of full test concentration, although inhibition did not cross the 50% threshold for IC50 determination.

61. Ethanolic extracts from E. purpurea were assessed for their ability to inhibit CYP 2E1 from human liver microsomes and an in vitro system baculovirus expression system (Raner et al., 2007). Fresh E. purpurea root was extracted with either 33% or 95% ethanol and 2µL of the extract was used in each 500 µL reaction. Eleven alkylamides were isolated from the Echinacea extract and individually tested for enzyme inhibition. The oxidation of p-nitrophenol was used to monitor the enzyme activity. The E. purpurea 95% ethanol extract yielded 30% inhibition in both human liver microsome and baculovirus derived CYP2E1. No inhibition was observed with the 33% ethanol extract. The authors noted that Echinacea extracts prepared using solvents with higher ethanol content have higher proportion of alkylamides, whilst the extracts prepared in ethanol/water mixtures have greater quantities of caffeic acid derivatives. The extracts with higher ethanol content exhibited higher inhibitory activity, suggesting that alkylamides rather than caffeic acid derivatives are likely inhibitory compounds in E. purpurea. No CYP2E1 inhibition was observed with two of the main caffeic acid derivatives, caftaric acid and chicoric acid, at concentrations up to 0.4 mM. The 11 individual alkylamides isolated from the 95% ethanol Echinacea extract showed inhibition against CYP2E1, with the four isobutylamides being most effective inhibitors achieving 40-60% inhibition at concentrations as low as 25 µM.

62. E. purpurea (L.) Moench Echinaforce extract was tested for its ability to inhibit baculovirus expressed CYP isoforms 1A2, 2C19, 2D6 and 3A4 in vitro (Modarai et al., 2010). A fluorescent based assay was used in three independent experiments run in duplicate and IC50 values were estimated by non-linear regression modelling (Hill model). Enzyme inhibition was observed at Echinaforce concentrations between 10 and 500 µg/mL, with IC50 values of 22 µg/mL (CYP3A4), 30 µg/mL (CYP1A2), 61 µg/mL (CYP2C19) and 69 µg/mL (CYP2D6). Nine commercially available Echinacea preparations, containing either E. purpurea, E. pallida or E. angustifolia, were screened for CYP3A4 inhibition. Their IC50 values varied by more than 50-fold, with the highest IC50 values of 824-1,812 µg/mL

obtained with E. purpurea expressed juice and the lowest IC50 values of 12.7-27.8 µg/mL with tinctures of E. purpurea and E. angustifolia. The authors quantified the alkylamide content of the preparations and reported that the total alkylamide content was positively associated with the ability of the preparations to inhibit CYP3A4.

63. The in vitro effects of E. purpurea on CYP1A2, CYP2D6 and CYP3A4 in human primary hepatocytes was investigated (Hellum et al., 2007). Echinagard commercially available preparation was used at concentrations 4.735, 47.35 and 473.5 µg/mL. The human primary hepatocytes were obtained from a 32-year-old male donor and all serology data was normal. The cultures were exposed to selected inducers positive controls (omeprazole for CYP1A2, rifampicin for CYP2D6 and CYP3A4) or the Echinacea preparation for 48, 72 and 96 hours. Basal enzyme activity in the absence of inducers was also determined. The activities of the enzymes were measured by HPLC analysis for the production of selected metabolites: phenacetin demethylation for CYP2A1, dextromethorphan O-demethylation for CYP2D6 and 6β-hydroxylation of testosterone for CYP3A4. E. purpurea showed general inhibitory activity against CYP1A2, CYP2D6 and CYP3A4 at all concentrations tested when compared to the basal enzyme activity.

64. The same authors also investigated the effects of Echinagard on the inhibitory potential of baculovirus expressed CYP2D6 (Hellum and Nilsen, 2007). The enzyme was incubated with dextromethorphan substrate for 25 minutes at 37°C prior to the addition of the Echinacea extract, positive control inhibitor quinidine or buffer/ethanol negative control. The dextrorphan, dextromethorphan O-demethylated metabolite, was extracted and quantified by HPLC for determination of the enzyme activity. Echinacea showed a maximum of 28% inhibition of CYP2D6 at the highest concentration tested (473.5 µg/mL).

65. The effect of E. purpurea on the P-glycoprotein (P-gp) was studied in human intestinal Caco-2 cells (Hansen and Nilsen, 2009). Commercially available E. purpurea preparation was used at a concentration range 0.0064-6.36 mg/mL, anticipated to cover an in vivo concentration range of the herb, estimated from a daily dose of 265 mg dried E. purpurea juice dissolved in 1 L of gastrointestinal or 56 L of total body fluid (0.27– 0.005 mg/mL). Digoxin (30 nM) was used as a substrate and verapamil as a control inhibitor. A significant linear dose-related decrease in the net digoxin flux was observed at Echinacea concentrations above 0.4 mg/mL. The Vmax (23.7 nmol/cm2 /h) and Km (385 µm) of the net digoxin flux decreased in the presence of E. purpurea in an uncompetitive fashion. The authors conclude that E. purpurea extracts, in compositions equal to those

present in commercial products, can interact with the P-gp mediated transport of digoxin in Caco-2 cells. They state that although the in vivo effects on systemic P-gp activity is probably limited, the potential of Echinacea to influence drug bioavailability cannot be excluded.

66. In a recent study, ethanolic extracts of 123 medicinal herbs, including Echinacea, were tested for their ability to induce CYP3A4 and CYP1A2 and their potential to inhibit P-gp (Husain et al., 2023). The Echinacea extract was prepared by extracting the dried plant material of E. angustifolia root in 95% ethanol, drying it and dissolving it in DMSO to prepare the stock solution. The cytochrome P450 inhibition assays were performed against baculovirus expressed human CYP3A4 and CYP2E1 by incubating the extracts with the enzymes and enzyme-specific substrates for 10 minutes and measuring the fluorescence of the product. Ketoconazole and α-naphthoflavone were used as positive controls for CYP3A4, and CYP1A2 isozymes, respectively. IC50 values were obtained from concentration-response curves generated by plotting percent inhibition versus tested concentrations. The P-gp inhibition was determined using a rhodamine-123 uptake assay in MDR1-MDCK cell line overexpressing P-gp. Various concentration of the Echinacea extract (12.5, 25, and 50 µg/mL), positive control (cyclosporin) and negative control (DMSO) were incubated with the cells for 90 minutes, lysed and the rhodamine-123 fluorescence was measured in the cell lysate. The E. angustifolia root extract was classed as a strong inhibitor of CYP3A4 with an IC50 value of 9 µg/mL, whilst no inhibition was observed against CYP1A2 up to the highest concentration tested (50 µg/mL). The extract exhibited a limited inhibition (>120% to <150%) in the rhodamine uptake assay and was therefore deemed to be a mild inhibitor of P-gp.

67. The effects of a standardised E. purpurea 60% ethanolic extract, containing 3.7% polyphenolic compounds expressed as caffeic acid, on the mRNA expression level of CYP1A1/2, CYP2D1/2, CYP3A1/2, CYP2E1, CYP2C6 in a rat model was investigated (Mrozikiewicz et al., 2010). Male Wistar rats were randomly divided into four groups from A to D (n = 10). Group A was treated once a day with 50 mg/kg oral E. purpurea extract for 3 days, and the control group B received a standard diet. Group C was treated with the same extract as group A, but for 10 days, and group D was used as control for group C. Total RNA was isolated from the rat liver tissue 16 hours after the administration of the last dose and level of gene expression in liver tissues was analysed by real-time quantitative PCR. The E. purpurea extract resulted in a potent inhibition of the expression of CYP3A1 (41%, $p < 0.05$) and CYP3A2 (25%, $p = 0.001$), and an induction of CYP1A1 (80%, $p = 0.01$) and CYP2D1 (40%, $p = 0.007$) after 10 days of treatment. The authors

speculate that the inhibition of the expression of CYP3A1/2 may translate to in vivo inhibition of human homologs CYP3A4/5 and influence the efficacy of chemotherapy as CYP3A4 is important in the metabolism of anti-cancer drugs. The conclusions of the study also state that the induction of CYP2D1, the homolog to the human CYP2D6, can lead to loss of pharmacological effect of prescription drugs in humans by reducing their plasma concentration.

68. A congress abstract describes the administration of different preparations of E. purpurea, E. angustifolia and E. pallida to a total of 216 rats assigned to different experimental groups (n=12) with various dosages, positive controls (ketoconazole, quinidine), or pure compounds (dodeca-2E,4E,8Z,10E/Z-tetraenoic acid isobutylamides; tetraenes) (Ardjomand-Woelkart et al., 2012). After treatment with different Echinacea preparations, probe drugs for CYP enzymes were orally administered before blood sampling: theophylline (CYP1A2), tolbutamide (CYP 2C9), dextromethorphan (CYP2D6) and midazolam (CYP3A4). Significant inhibition of CYP1A2 were observed with some Echinacea preparations, weak inhibition of CYP2D6, but no inhibition of CYP3A4 and CYP2C9.

Human studies

69. The effects on E. purpurea root on CYP1A2, 2C9, 2D6 and 3A were assessed in 12 healthy volunteers (6 men and 6 women) aged 31 ± 6 years in a 2 week open-label study (Gorski, 2004). Participants were non-smokers, had no significant medical conditions and had not used herbal products in the 6 months prior to the start of the study. They were instructed not to consume any alcohol, caffeine, grapefruit products, apple or orange juice, cruciferous vegetables or charbroiled meats for at least one week before and until the end of the study. Single dose of CYP probe drugs were administered before and after a short course of a commercially available E. purpurea root extract (Nature’s Bounty) taken orally at 1,600mg/day for 8 days. This preparation contains greater than 1% phenols (caftaric acid, chlorogenic acid, echinacoside and chicoric acid). The following probes were used: caffeine (CYP1A2), tolbutamide (CYP2C9), dextromethorphan (CYP2D6), and midazolam (hepatic and intestinal CYP3A4) and their plasma-concentration profiles were determined before and after Echinacea supplementation.

70. The administration of Echinacea at 400 mg 4 times a day for 8 days was well tolerated, and no adverse events were reported by the volunteers or observed by the researchers. The Echinacea significantly reduced the oral clearance of caffeine, the CYP1A2 probe, by 27% (p=0.049) from 6.6 ± 3.8 L/h to

4.9 ± 2.3 L/h, indicating inhibition of the CYP1A2 enzyme and increased the maximum serum concentration of caffeine by 30%. Interindividual variability was observed as 2 men showed greater than 50% reduction in caffeine clearance. The authors concluded that the modest change in the clearance of compounds metabolised by CYP1A2 is considered clinically significant as this can lead to increased toxicity of narrow therapeutic window drugs such as theophylline, which is a substrate for CYP1A2. They also speculated that other drugs metabolised by CYP1A2 such as cyclobenzaprine, tacrine, and clozapine can be affected by Echinacea coadministration (Gorski, 2004).

71. The systemic clearance of intravenously administered midazolam, CYP3A4 probe, significantly increased by 34% (from 32 ± 7 L/h to 43 ± 16 L/h, p=0.003) and the AUC decreased by 23% (p=0.24). The administration of Echinacea resulted in a 43% increase (from 0.23 to 0.33, p =0.028) in the oral bioavailability of midazolam despite an observed reduction in its hepatic availability (15%, p =0.006). The authors concluded that this increase in oral bioavailability is mediated by a 2-fold increase (from 0.33 to 0.61, p=0.015) in the intestinal availability of midazolam, suggesting that Echinacea inhibits the intestinal CYP3A4 isoform. The changes in the intestinal and hepatic availability balanced out, resulting in an oral clearance and AUC of midazolam unchanged by the Echinacea dosing.

72. Echinacea administration also significantly reduced the oral clearance of tolbutamide, the CYP2C9 probe, from 0.81 ± 0.18 L/h to 0.72 ± 0.19 L/h (11%, p = 0.001), but had no significant effects on the maximum serum tolbutamide concentration reached. Two individuals (1 man and 1 woman) had a 25% or greater reduction in the oral clearance of tolbutamide. These results indicate an inhibition of CYP2C9 by Echinacea, but this was not considered to be clinically important as the geometric mean and the 90% confidence intervals (CIs) for AUC (area under the curve), oral clearance, and maximum concentration were within the default no-effect boundaries of 80% to 125% as per the criteria established by the Food and Drug Administration (FDA) in its industry guidance concerning in vivo drug interaction studies.

73. Echinacea dosing did not significantly influence the pharmacokinetic parameters (AUC, clearance, maximum serum concentration and half-life) of the CYP2D6 probe dextromethorphan, suggesting that co-administration of Echinacea is not likely to alter the metabolism of drugs metabolised by CYP2D6 (Gorski, 2004). This aligns with the results of another human study investigating the effects of botanical supplements on the CYP2D6 activity in 16 healthy volunteers,

which reported that Echinacea did not have significant inhibitory effect on CYP2D6 (Gurley et al., 2008). All the subjects in that study were confirmed to be extensive CYP2D6 metabolisers, were nonsmokers and were not taking any botanical supplements or prescription medication. E. purpurea (Gaia Herbs, standardised to contain 2.2 mg isobutylamides per capsule) was administered for 14 days at 267 mg, three times daily. The CYP2D6 activity was assessed using 8-hour debrisoquine urinary recovery ratios using fluorescence detection.

74. Another human study with 12 healthy volunteers (6 men, 6 women) investigated the effects of E. purpurea (800 mg, twice daily) for 28 days on CYP1A2, CYP2D6, CYP2E1 and CYP3A4 phenotypes (Gurley et al., 2004). The composition of the Echinacea preparation was analysed using HPLC and it was determined that it contained 13.7 mg chicoric acid per capsule, providing a daily dose of 43.8 mg chicoric acid. All subjects were extensive metabolizers of CYP2D6 as confirmed by debrisoquin urinary recovery screenings. The mean age of the participants was 25 ± 3.0 years, they were nonsmokers, healthy and did not use botanical supplements. All subjects were asked to abstain from alcohol, caffeine, fruit juices, cruciferous vegetables, and charbroiled meat throughout the study. Caffeine and midazolam were used for CYP1A2 and CYP3A4 probes. To avoid potential interference from midazolam and caffeine, CYP2E1 and CYP2D6 phenotypes were assessed 24 hours later by administering oral chlorzoxazone (CYP2E1) and debrisoquin (CYP2D6). The CYP modulatory capability of Echinacea was evaluated by comparing individual differences in metabolite/parent serum ratios before and at the end of 28 days of supplementation.

75. No serious adverse events occurred during the course of the study; one subject experienced a mild rash develop while taking Echinacea. In this study, E. purpurea given over 28 days did not significantly change the activities of CYP3A4, CYP2E1, and CYP2D6 as estimated by comparing the phenotype ratios before and after treatment. Co-administration of E. purpurea caused an approximately 13% decrease in the ratio of paraxanthine/caffeine, suggesting that there was a possible inhibitory effect on CYP1A2 enzyme. However, the difference was not statistically significant and the authors did not think it was clinically relevant (Gurley et al., 2004).

76. The herb-drug interaction between E. purpurea and lopinavir-ritonavir was investigated in 13 healthy volunteers (8 men, 5 women) aged between 18-50 years (Penzak et al., 2010). Patients were healthy, had negative HIV test, were nonsmokers and were not allowed to take any medication within 30 days of study participation. Subjects were given single oral midazolam and fexofenadine to act as CYP3A4 and P-gp probes respectively. Seven to 28 days after midazolam and

fexofenadine administration, subjects began taking lopinavir/ritonavir 400 mg/100 mg twice daily with meals. 15 days after the antiretroviral therapy initiation, E. purpurea fresh liquid extract 8:1 softgel capsules from a single batch (Echinamide Natural Factors) was initiated at 500 mg three times daily (1,500 mg daily dose) for 28 days. The extract formulation contained standardized amounts of alkylamides, polysaccharides, and chicoric acid. The patients continued taking the lopinavir/ritonavir for a further 14 days (total 29.5 days) with the last 2 weeks of the study consisting only of Echinacea dosing. At the end of the study, patients returned for repeat fexofenadine and midazolam administration. Neither lopinavir nor ritonavir were significantly influenced by the 2-week Echinacea co-administration. The AUC of midazolam was significantly decreased ($p= 0.008$), whilst its clearance was significantly increased ($p=0.02$) after the E. purpurea administration suggesting induction of the CYP3A4-mediated metabolism of midazolam. No effect on the fexofenadine pharmacokinetic parameters was observed. The authors explain the lack of change in the pharmacokinetic parameters of the CYP3A4 substrates lopinavir and ritonavir with ritonavir being a potent intestinal and hepatic CYP3A4 inhibitor, therefore masking any enzyme induction effects of Echinacea.