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Essential oils of culinary herbs and spices activate PXR and induce CYP3A4 in human intestinal and hepatic *in vitro* models

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ABSTRACT

Essential oils (EOs) are extensively used in food industry, gastronomy and alternative medicine. They are multicomponent mixtures of bioactive compounds; hence, their potential for food-drug interactions is substantial. In this study, we investigated the effects of 31 EOs of culinary herbs and spices on the transcriptional activity of pregnane X receptor (PXR) and expression of cytochrome P450 3A4 (CYP3A4), using human intestinal and hepatic *in vitro* models. All tested EOs activated PXR in intestinal LS180 cells transiently transfected with PXR, as revealed by a reporter gene assay. Consistently, all EOs induced *CYP3A4* mRNA expression in PXR-transfected LS180 cells, primary human hepatocytes and wild-type hepatic progenitor HepaRG cells. EO-mediated induction of *CYP3A4* mRNA expression was nullified in PXR-knock out HepaRG cells, suggesting the involvement of PXR in these effects. Collectively, we showed that EOs of culinary herbs and spices might be common activators of PXR and inducers of CYP3A4 at doses present in foods, thereby, they might have a potential for food-drug interactions. Follow-up studies are warranted to identify the bioactive constituents in the tested EOs.

1. Introduction

Various natural products, including essential oils (EOs) of culinary herbs and spices, are extensively used in food industry and gastronomy – to flavour, preserve, and colour products. Since EOs are multicomponent mixtures of structurally diverse chemicals, dietary intake of EOs involves consumption of tens to hundreds biologically active substances of natural origin. According to recipes in cookery books, one drop of EOs is an equivalent of one tea spoon of dried herbs or spices. The recommendation is to use one drop of EOs *per* large bowl, pan, pot, or dish. Considering an approximate drop volume and volumetric mass density of EOs, the concentration of EOs in ingested foods ranges from 35 to 55 μg/mL. Given the frequency and extent of EOs consumption, chemical complexity and the ingested doses of EOs, they might have a potential to cause food-drug interaction. Food-drug interactions occur by two main mechanisms, (i) inhibition of catalytic activity of drug-metabolizing enzymes, or function of drug transporters, or (ii) activation or inactivation of transcriptional regulators of xenobiotic-metabolizing enzymes. The pivotal transcriptional regulators of xenobiotic-metabolizing enzymes, sometimes referred to as xenosensors, include aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR), and pregnane X receptor (PXR) (Pascussi et al., 2008). Recently, we investigated the effects of EOs of culinary herbs and spices on the AhR-CYP1 A1 signalling pathway (Bartonkova and Dvorak, 2017). However, data on the effects of EOs on the PXR-CYP3A4 pathway are scarce. Besides the roles of PXR in the control of drug metabolism, PXR has been showed to be involved in the regulation of lipid and carbohydrate metabolism (Hakkola et al., 2016), immune responses (Venkatesh et al., 2014), and pathogenesis of cancer (Planque et al., 2016), diabetes (Hukkanen et al., 2014), and inflammatory bowel disease (Garg et al., 2016). Therefore, there has been a need to investigate the effects of EOs on the PXR-CYP3A4 signalling pathway. Cloves and thyme extracts activated PXR, which correlated with the activation of CYP3A4 promoter for thyme extract but not for clove extract (Kluth et al., 2007). Ligustilide, a major constituent of lovage EO, was identified as a PXR agonist in a reporter gene assay (Yu et al., 2011). Carnosic acid, a constituent of rosemary EO, induced *CYP2B6* and *CYP3A4* mRNA expression and enzyme activities in human hepatocytes (Dickmann et al., 2012); additionally, it activated PXR and induced *CYP3A4, UGT1A3,* and ATP-binding cassette sub-family B member (*ABCB1*) expression in LS180 cells (Seow and Lau, 2017). Moreover, a constituent of ginger, 6-gingerol, induced *CYP3A4* mRNA expression in

Abbreviations: AhR, aryl hydrocarbon receptor; EO, essential oil; EtOH, ethanol; CAR, constitutive androstane receptor; GR, glucocorticoid receptor; PXR, pregnane X receptor; RIF, rifampicin; VDR, vitamin D receptor

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HepG2 cells (Li et al., 2013). Several constituents of black pepper have been showed to affect the PXR-CYP3A4 pathway. Piperine induced CYP3A4 and multidrug resistance protein 1 (MDR1) expression in human hepatocytes, intestinal cells, and in a mouse model (Wang et al., 2013; Hu et al., 2015). Nigramide J was shown to act as a dual ligand of CAR and PXR, as the inverse agonist and agonist, respectively (Kanno et al., 2014a). Nigramide C activated PXR and induced CYP3A4 mRNA and protein expression in hepatoma cells and primary human hepatocytes (Kanno et al., 2014b).

In the current study, we investigated the effects of 31 EOs of culinary herbs and spices on the transcriptional activity of PXR and expression of CYP3A4, using human intestinal and hepatic *in vitro* models. We showed that these EOs might be common activators of PXR and inducers of CYP3A4 at doses present in foods, thereby, they might have a substantial potential for food-drug interactions.

2. Materials and methods

2.1. Chemicals

EOs of dill (fruits), tarragon (flowering tops), caraway (seeds), cinnamon (bark), coriander (leaves), cumin (fruits), turmeric (roots), lemongrass (flowers), cardamom (fruits), cloves (buds), fennel (flowering tops); star anise (fruits), jasmine (blossoms), juniper (twigs and berries), bay leaf (leaves), lovage (roots); verveine (leaves), cornmint (flowers), spearmint (flowers), peppermint (flowers), nutmeg (fruits), basil (flowering tops), oregano (flowering tops), marjoram (flowering tops), black pepper (fruits), rosemary (flowering tops), sage (flowering tops), thyme (flowering tops), vanilla (oleoresin), and ginger (rhizomes) were purchased from Pranarôm (Ghislenghien, Belgium). EO of anise, rifampicin (RIF), and charcoal-stripped foetal bovine serum were purchased from Sigma Aldrich (Prague, Czech Republic). Detailed information on analyses of purity, composition and origin of EOs was published elsewhere (Bartonkova and Dvorak, 2017). Hygromycin B was obtained from SantaCruz Biotechnology (Santa Cruz, CA, USA). Reporter lysis buffer and FuGENE® HD transfection reagent were obtained from Promega (Hercules, CA, USA). All other chemicals were of the highest quality commercially available.

2.2. Cell cultures

Human Caucasian colon adenocarcinoma cells LS180 (ECACC No. 87021202) were purchased from the European Collection of Cell Cultures (ECACC). Cells were cultured in supplemented Dulbecco's modified Eagle's medium (DMEM) medium, according to the supplier's recommendations.

Primary human hepatocytes cultures Hep200551, Hep200553 and Hep200554 were obtained from Biopredic International. All three liver donors were males, negative for virology (hepatitis B/C, HIV-1/2), non-diabetics and non-smokers. Additional information on liver donors is shown in table-insert in Fig. 5.

PXR knock-out (PXR-KO) and control 5 F Clone HepaRG hepatic progenitor cells were obtained from Sigma Aldrich (Prague, Czech Republic) and cultured according to the manufacturer's instructions. Cells were maintained at 37 °C and 5% CO_2 in a humidified incubator.

2.3. Reporter gene assay

Transiently transfected LS180 human colon adenocarcinoma cells were used for assessment of PXR transcriptional activity. A chimera p3A4-luc reporter construct containing the basal promoter (−362/+53) with a proximal PXR response element and distal xenobiotic responsive enhancer module (−7836/−7208) of the CYP3A4 gene 5′-flanking region inserted into thepGL4.10 reporter vector was used (Pavek et al., 2010). The reporter plasmid was transiently transfected to LS180 cells by lipofection (FuGENE® HD transfection reagent). Cells were seeded in 96-well tissue culture plates and following 24 h of stabilization, they were incubated for 24 h with tested EOs and vehicle (ethanol; EtOH; 0.1% v/v) in the presence (agonist mode) or absence (antagonist mode) of RIF (10 μM). Then, the cells were lysed, and luciferase activity was measured using a Tecan Infinite M200 Pro plate reader (Schoeller Instruments, Czech Republic). A substrate containing 0.5 mM D-luciferin, 0.5 mM Adenosine 5′-triphosphate (ATP) and 0.2 mM Coenzym A was used. Cell viability was determined by the conventional 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

2.4. Determination of mRNA expression and qRT-PCR

Cells were seeded in 12-well plates in concentration 3.5×10^5 cells per well and following the stabilization, they were treated with tested EOs for 24 h. The concentrations of EOs used for mRNA analyses were selected based on cytotoxicity assay in LS180 cell line published previously as well as on the highest induction observed in reporter gene assay. Following the incubation, cells were washed with PBS and total RNA was isolated using 0.5 ml of TRI Reagent® (Sigma Aldrich, Prague, Czech Republic). The total RNA concentration and purity was determined by NanoDrop™ Lite spectrophotometer by ThermoScientific (Waltham, MA, USA). cDNA was synthesized from 1000 ng of total RNA using M-MuLV Reverse Transcriptase and Random Primers 6 by New England Biolabs (Ipswith, MA, USA) and diluted in 1:4 ratio by PCR grade water. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) was performed using LightCycler ® 480 Probes Master on a Light Cycler ® 480 II apparatus (Roche Diagnostic Corporation). The levels of *CYP3A4*, *CYP2B6*, *MDR1*, and *GAPDH* mRNAs were determined using Universal Probes Library (UPL; Roche Diagnostic Corporation) probes and primers as described in Table 1. The following protocol was used: an activation step at 95 °C for 10 min was followed by 45 cycles of PCR (denaturation at 95 °C for 10 s; annealing with elongation at 60 °C for 30 s). The amplicon identification by melting curve or sequencing is not necessary since the specificity of the qPCR reaction is ensured by the combination of UPL probe and primer pair designed by online tool Assay Design Center by Roche. Measurements were performed in triplicates. The data were processed by the delta-delta method and they were normalized *per GAPDH* as an endogenous gene.

2.5. Statistical analysis

In the reporter gene assays and qRT-PCR mRNA analyses, one-way analysis of variance (ANOVA) followed by Dunnett's test was applied to determine significantly different results over negative control (0.1%

Table 1
Primer sequences with appropriate Universal Probes Library (UPL) numbers.

Gene	Forward primer sequence	Reverse primer sequence	UPL no.
GAPDH	CTCTGCTCCTCCTGTTCGAC	ACGACCAAATCCGTTGACTC	60
CYP3A4	TGTGTTGGTGAGAAATCTGAGG	CTGTAGGCCCCAAAGACG	38
CYP2B6	TTCACTCATCAGCTCTGTATTCG	GCCCCAGGAAAGTATTTCAA	106
MDR1	CCTGGAGCGGTTCTACGA	TGAACATTCAGTCGCTTTATTTCT	147