

The Effect of (-)-Linalool on the Metabolic Activity of Liver CYP Enzymes in Rats

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Summary

(-)-Linalool is the major floral scent occurring mainly in families *Lamiaceae*, *Lauraceae* and *Rutaceae* and is the main active compound of lavender oil. The purpose of this study was to reveal the influence of subchronic systemic treatment with (-)-linalool on the metabolic activity of CYP2A, 2B, 2C6, 2C11 and 3A in rat liver microsomes (RLM). The second aim was to reveal possible inhibitory effect of (-)-linalool on CYP2C6 *in vitro*. Wistar albino male rats were treated with (-)-linalool intragastrically at the doses of 40, 120, and 360 mg/kg/day for 13 days. Treatment with (-)-linalool at the dose of 360 mg/kg increased the metabolic activity of CYP2A assessed with testosterone as a probe substrate. (-)-Linalool showed weak competitive inhibition of CYP2C6 in rat liver microsomes, with IC_{50} of 84 μ M with use of diclofenac as a probe substrate.

Key words

(-)-Linalool • Rat liver microsomes • CYP450 • Metabolic activity

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Introduction

Linalool is a monoterpenic alcohol present either in monocotyledonous and dicotyledonous plants. There were identified two enantiomers in plants. (3S)-(+)-Linalool, also known as coriandrol, is the main component of essential oils of *Coriandrum sativum* seeds. (3R)-(-)-Linalool, known as licareol, is the main component of essential oil of *Lavandula officinalis*

flowers. Except of the distribution, the enantiomers differ in a fragrance and biological activity. (+)-Linalool is described as sweet, floral and herbaceous while (-)-linalool as a woody, lavender-like aroma (Aprotosoae *et al.* 2014). The subject of this study is (-)-linalool, the main active substance of *Lavandula spp.* which is used in traditional medicine to treat anxiety or mild depression (EMA/HPMC 2012). Many biological activities of (-)-linalool have been proven, for example anti-inflammatory (Peana *et al.* 2002), antimicrobial (Park *et al.* 2012) and antioxidant activity (Liu *et al.* 2012). Many studies have also shown diversity of other pharmacological properties such as sedative (Sugawara *et al.* 1998), anxiolytic (Souto-Maior *et al.* 2011), anticonvulsant (Elisabetsky *et al.* 1999), analgesic (Li *et al.* 2016) and local anesthetic activity (Zalachoras *et al.* 2010). Food supplements containing (-)-linalool whose efficacy in treatment of anxiety disorders was confirmed by clinical trial (Kasper *et al.* 2010) are available in Central Europe. Furthermore, lavender oil preparation has been licensed in Germany as a herbal medicinal product for the treatment of restlessness in anxiety disorders (Uehleke *et al.* 2012).

According to the increasing popularity of alternative medicine and rising use of herbal preparation, knowledge on pharmacodynamic and pharmacokinetic herbal-drug interactions are needed and required by state authorities in the registration procedure of herbal medication. The aim of this study was to evaluate the influence of subchronic administration of (-)-linalool on the metabolic activity of liver CYP2A, CYP2B, CYP2C6, CYP2C11 and

CYP3A enzymes in rats and to determine their IC_{50} for a purpose to predict possible herbal-drug interactions.

Methods

Animals

Male Wistar albino rats (280±20 g, 8 weeks old) were housed in groups of 5 under standard laboratory conditions (12/12 h light-dark regime at the temperature 22±2 °C and room humidity 55±5 %). Water and pelleted diet were provided *ad libitum*. All experiments were performed in accordance with the Czech act No. 246/1992 and with the approval of both the local and national Czech Central Commission for Animal Welfare.

Experimental design

After 5 days of acclimatization, rats were randomly divided into four groups per 10 animals. Animals were treated intragastrically with (-)-linalool dissolved in a 1 % Tween 20 at the doses of 40, 120 and 360 mg/kg/day. The control group was administered with appropriate volume of vehicle (5 % glucose + 1 % Tween 20, 1 ml/kg). The administration was carried out for 13 consecutive days. Animals were sacrificed by decapitation 24 h after the last drug administration and the liver was sampled and frozen until microsomes isolation.

Chemicals

(-)-Linalool, NADP, glucose-6-phosphate, glucose-6-phosphate-dehydrogenase, $MgCl_2 \cdot 6 H_2O$, EDTA, KH_2PO_4 , Na_2HPO_4 , HCl, prednisone, ibuprofen, testosterone, diclofenac, Tween 20, KCl, Tris, 4'-hydroxydiclofenac and sucrose were provided by Sigma-Aldrich (St. Louis, MO, USA). Metabolites of testosterone (2 β -, 2 α -, 7 α -, 6 β -, 16 α -, 16 β - hydroxyl-testosterone) were purchased from Steraloids Inc. (Newport, RI, USA). All the organic solvents were of HPLC purity (acetonitrile, methanol, dichloromethane and diethyl ether) and were supplied by Lach-Ner (Neratovice, CZ).

Preparation of RLM

Microsomes were isolated from 3 grams of liver tissue of individual animals by differential ultracentrifugation (19000 g for 20 min and 2×105000 g) in 20 mM Tris/KCl and finally diluted in 0.25 M Tris/sucrose buffer (pH=7.4). The total protein

content in the microsomal preparations was assessed according to Lowry *et al.* (1951) method using the bovine serum albumin as a standard. Total CYP content was assessed by CO-difference spectroscopy according to Omura *et al.* (1964) method.

Determination of Cytochrome P450 activity in RLM

The activities of CYP2A, 2B, 2C11 and 3A were assessed by measuring the rate of testosterone hydroxylation in positions: 7 α (CYP2A), 16 β (CYP2B), 2 α and 16 α (CYP2C11), 2 β and 6 β (CYP3A) according to the modified method of Wójcikowsky *et al.* (2008) with slight modifications (Turjap *et al.* 2014) as was described previously (Dovrtělová *et al.* 2015). The activity of CYP2C6 was assessed by measuring the rate of 4'-hydroxylation of diclofenac. Incubation mixture of final volume 0.5 ml containing phosphate buffer (50 mM, pH=7.4), EDTA (1.1 mM), NADP (1.2 mM), glucose-6-phosphate (4.4 mM), $MgCl_2 \cdot 6 H_2O$ (3.2 mM), glucose-6-phosphate-dehydrogenase (0.5 U in 0.5 ml), 50 μ l of RLM (1 mg/ml of protein) and diclofenac (100 μ M). Drugs' effects were evaluated in the range of linear dependence of the product formation on time and concentrations of substrates. The reaction was carried out at 37 °C on horizontal vortex at 180 rpm and stopped after 20 min by addition of 50 μ l of ice cold methanol and cooling down on the ice. Metabolites formed during activity assays were measured by HPLC (Shimadzu LC-10) with the DAD detector (Shimadzu SPD-M10AVP). Internal standard (ibuprofen) was added to the analytes and 4'-hydroxydiclofenac was extracted by 10 min of vortexing with diethyl ether (4 ml). The residue obtained after evaporation of extracts was dissolved in 250 μ l of mobile phase. An aliquot of 20 μ l was injected into the HPLC system and the mobile phase was used in following isocratic mode: 57:43 v/v KH_2PO_4 (20 mM, adjusted to pH=2.8)/acetonitrile. The flow rate was 0.55 ml/min. Analytical column (Kinetex C18 2.6 μ , 150 x 4.6 mm) was purchased from Phenomenex (Torrance, CA, USA). The absorbance was measured with DAD detector at the wavelength 225 nm (ibuprofen) and 276 nm (diclofenac and 4'-hydroxydiclofenac). Metabolic activities of all CYP enzymes were expressed as the metabolite molar concentration/min/mg of total protein in RLM. Inhibitory potency of (-)-linalool on CYP2C6 metabolic activity was evaluated in RLM incubated with diclofenac after 10 min of pre-incubation with (-)-linalool (1 μ M – 1 mM).

Data analysis

Data were statistically evaluated with the Statistica 12 software (StaSoft, Inc. 2013) using non-parametric Kruskal-Wallis test. Results were regarded as statistically significant when $p \leq 0.05$. IC_{50} value was determined through nonlinear regression of relative reaction rate at single substrate concentration (25 μ M) and 0.25 mg/ml of total protein in the presence of varying inhibitor concentrations (1 nM – 1 mM) and calculated by using SigmaPlot (SPSS, Inc., Chicago).

Results

Neither total protein content nor total CYP

content in RLM were affected by any of treatments (Fig. 1). Treatment with (-)-linalool showed insignificant, dose dependent tendency with increase in the rate of testosterone 7 α -(CYP2A), 6 β -(CYP3A), and 16 β -hydroxylation (CYP2C11) but only dose 360 mg/kg exhibited statistically significant increase in the rate of 7 α -hydroxylation of testosterone (CYP2A) (Fig. 2). (-)-Linalool exhibited also dose-dependent trend on the rate of 4'-hydroxylation of diclofenac (CYP2C6) which did not reach statistical significance at any of treatment regimes (Fig. 2). Within 1 nM – 1 mM (-)-linalool exhibited weak competitive inhibition of CYP2C6, with IC_{50} of 84 μ M (Fig. 3).

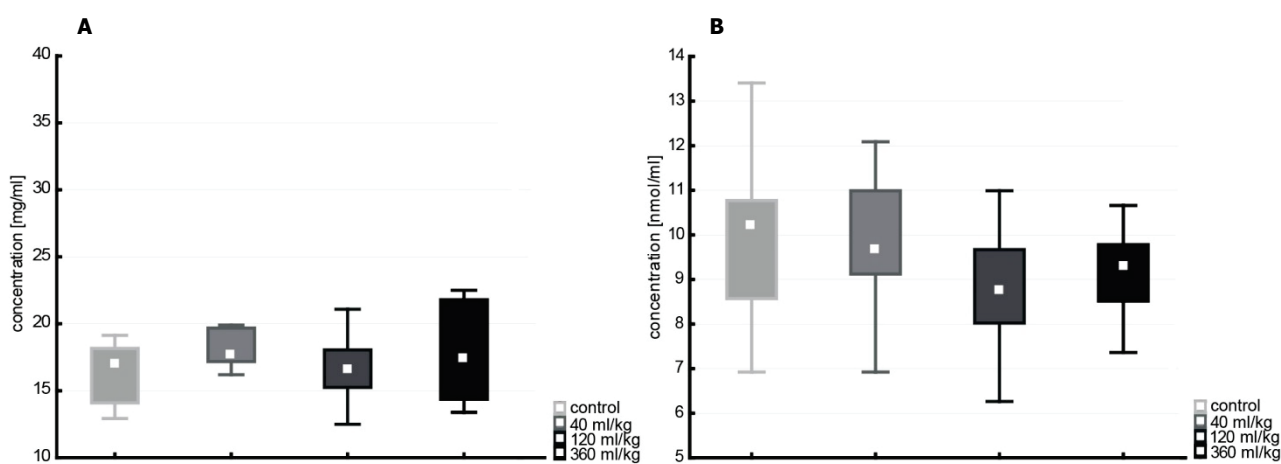


Fig. 1. The effect of subchronic administration of (-)-linalool on total protein (A) and total CYP content (B) in RLM. Values expressed as box plots with median (box 25 – 75 %, whiskers min – max outliers).

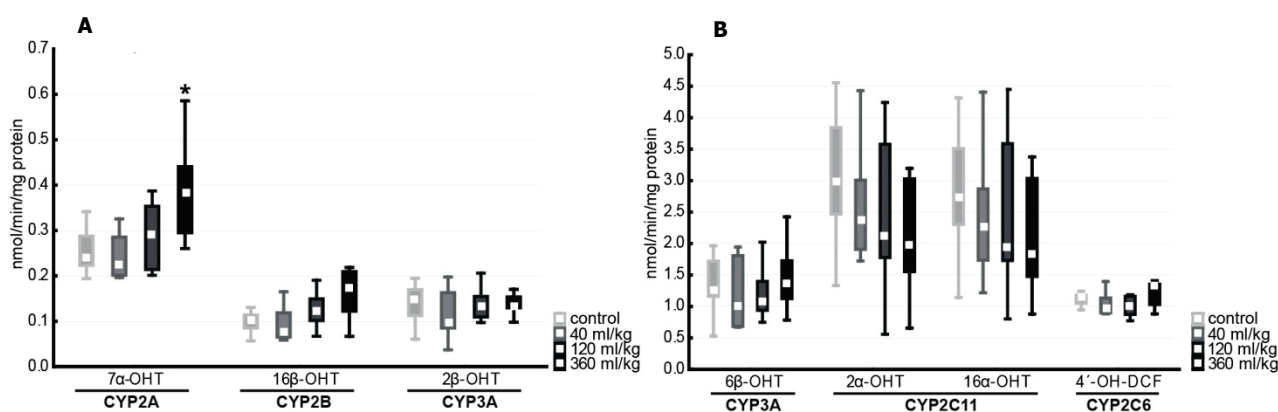


Fig. 2. The metabolic activity of selected CYP enzymes in RLM after systemic subchronic administration of (-)-linalool expressed as nmol/min/mg of total protein (OHT = hydroxytestosterone, 4'-OH-DCF = 4'-hydroxydiclofenac). All values are expressed as box plots with median (box 25 – 75 %, whiskers min-max without outliers). Statistical significance with respect to the control group is indicated with * $p \leq 0.05$.

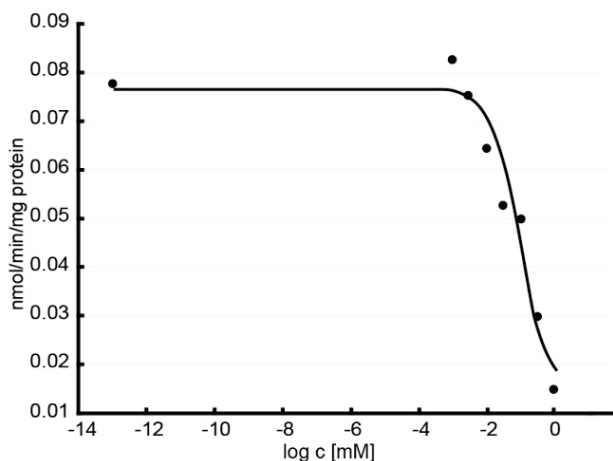


Fig. 3. Effect of (-)-linalool on the *in vitro* biotransformation of diclofenac to 4'-hydroxydiclofenac by RLM with NADPH generating system. A fixed concentration of diclofenac (25 μ M) was incubated with various concentrations of (-)-linalool (1 nM – 1 mM). The IC₅₀ value was determined by nonlinear regression analysis.

Discussion

The objective of this study was to assess *in vitro* metabolic activity of selected CYP enzymes in RLM after subchronic administration of (-)-linalool to rats. The metabolic activity of CYP2A, CYP2B, CYP2C11 and CYP3A was assessed with the use of testosterone and the metabolic activity of CYP2C6 with diclofenac as a probe substrate. The results from the study demonstrate that dose 360 mg/kg of (-)-linalool significantly increased the rate of 7 α -hydroxylation of testosterone, which correspond to increased metabolic activity of CYP2A. In rat, the CYP2A family includes CYP2A1, CYP2A2 occurring in hepatic tissue and CYP2A3 occurring in lungs. The rat CYP2A show about 60 % homology in amino acid sequence to human CYP2A6 (Martignoni *et al.* 2006). There are only few drugs which are predominantly metabolized *via* CYP2A (coumarin, nicotine), whereas for the most of xenobiotic substrates CYP2A represents only minor pathway of their biotransformation (halothane, methoxyflurane, valproic acid etc.). However, CYP2A displays restricted substrate specificity, it was found that can metabolically activate a number of carcinogens including nitrosamines and

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aflatoxins (Raunio *et al.* 2008).

To the best of our knowledge, up to date there is no study which tested the effect of (-)-linalool on CYP enzymes. Doroshenko *et al.* (2013) evaluated effect of lavender oil preparation in 16 humans with result that 160 mg/day of lavender oil preparation had no clinically relevant effect on CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 enzymes *in vivo*. Kasper *et al.* (2013) reviewed 7 clinical trials investigating the anxiolytic efficacy of lavender oil preparation, which generally confirmed that doses 80 – 160 mg/day have the anxiolytic effect in patients with subsyndromal anxiety and generalised anxiety disorder. The doses tested by ours in the experiment with subchronic administration of (-)-linalool to rats were much higher, and therefore we assume that possible influence of linalool on drug metabolizing enzymes is not clinically relevant.

Conclusions

In the light of our results, (-)-linalool could be considered as safe anxiolytic/antidepressant adjuvant to conventional treatment in the light of drug-drug interactions. For confirmation of our results, it is necessary to replicate our results in other experimental system, e.g. human liver microsomes or immobilized CYP microreactor system (Schejbal *et al.* 2016) which could enable repeated use of human CYP enzymes with series of CYP probe substrates.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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