Toxicology in Vitro 28 (2014) 693–699

Contents lists available at ScienceDirect

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Palmatine activates AhR and upregulates CYP1A activity in HepG2 cells but not in human hepatocytes

Jiri Vrba [a,*], Marika Havlikova [b], Denisa Gerhardova [a], Jitka Ulrichova [a,b]

[a] Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacky University, Hnevotinska 3, Olomouc 77515, Czech Republic
[b] Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University, Hnevotinska 3, Olomouc 77515, Czech Republic

ARTICLE INFO

Article history:
Received 18 November 2013
Accepted 17 February 2014
Available online 27 February 2014

Keywords:
Alkaloid
Palmatine
Cytochrome P450
CYP1A1
Aryl hydrocarbon receptor
Herb–drug interactions

ABSTRACT

The protoberberine alkaloid palmatine is present in preparations from medicinal plants such as *Coptis chinensis* and *Corydalis yanhusuo*. This study examined whether palmatine affects the expression of cytochromes P450 (CYPs) 1A1 and 1A2 in primary cultures of human hepatocytes and human hepatoma HepG2 cells grown as monolayer or spheroids. Gene reporter assays showed that palmatine significantly activated the aryl hydrocarbon receptor (AhR) and increased the activity of *CYP1A1* gene promoter in transiently transfected HepG2 cells. In HepG2 monolayer culture, palmatine also significantly increased mRNA and activity levels of CYP1A1, albeit with considerably less potency than 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, a prototypical CYP1A inducer. On the other hand, CYP1A activity was not significantly elevated by palmatine in HepG2 spheroids. Moreover, palmatine induced mild or negligible changes in CYP1A1 and CYP1A2 mRNA expression without affecting CYP1A activity levels in primary human hepatocytes. It is concluded that palmatine activates the AhR-CYP1A pathway in HepG2 monolayer, while the potential for CYP1A induction is irrelevant in cell systems which are closer to the *in vivo* situation, i.e. in HepG2 spheroids and primary cultures of human hepatocytes. Possible induction of CYP1A enzymes by palmatine *in vivo* remains to be investigated.

1. Introduction

The protoberberine alkaloid palmatine (Fig. 1) is one of the main bioactive components of preparations from medicinal plants such as *Coptis chinensis* (Ma and Ma, 2013) and *Corydalis yanhusuo* (Kim et al., 2011). Herbal products containing palmatine and other protoberberine alkaloids are mainly used in traditional medicine in China, Korea and India (Khan et al., 2013) for their antimicrobial, antiinflammatory, antidiabetic, hepatoprotective and analgesic effects (Ma and Ma, 2013; Yi et al., 2013). Palmatine alone has been shown, for instance, to attenuate galactosamine/lipopolysaccharide-induced hepatic failure in mice (Lee et al., 2010) and to protect the heart from ischemia/reperfusion injury in rats (Kim et al., 2009). At the molecular level, the biological activity of palmatine is presumably associated with its ability to interact with proteins (Khan et al., 2013) and nucleic acids (Bhadra and Kumar, 2011). For example, palmatine has been reported to inhibit enzymes including reverse transcriptase (Sethi, 1983) and acetylcholinesterase (Huang et al., 2012), and, to modulate expression of genes such as those encoding calmodulin 1, Janus kinase 2 and inositol polyphosphate multikinase (Suzuki et al., 2011).

Herbal products are usually available without prescription, and hence patients may combine herbal remedies and conventional drugs without medical advice. It is therefore necessary to identify possible drug-drug and/or herb–drug interactions which can arise from the inhibition or induction of drug-metabolizing enzymes, particularly cytochrome P450 (CYP) enzymes (Thummel and Wilkinson, 1998). The potential for herb–drug interactions is generally rather high since phytopreparations contain complex mixtures of pharmacologically active compounds. Palmatine has been found to inhibit the activity of several human CYP enzymes including CYP1A1, CYP1B1 (Lo et al., 2013), CYP2D6 (Han et al., 2011) and CYP3A4 (Su et al., 2007). The effect of palmatine on the expression of drug-metabolizing enzymes has not been studied to date. Nonetheless, it has been shown that berberine (Fig. 1), an alkaloid structurally related to palmatine, is an activator of the aryl hydrocarbon receptor (AhR) (Vrzal et al., 2005), a ligand-dependent transcription factor that regulates the expression of CYP1A1, CYP1A2 and CYP1B1 (Nebert et al., 2004). CYP1A1 and CYP1B1 are primarily extrahepatic enzymes, while CYP1A2 is constitutively expressed in human liver (Chang and Waxman, 1998) where it metabolizes approximately 5% of therapeutic drugs (Ingelman-Sundberg and

* Corresponding author. Tel.: +420 585632310; fax: +420 585632302.
E-mail address: vrbambv@seznam.cz (J. Vrba).

http://dx.doi.org/10.1016/j.tiv.2014.02.008
0887-2333/

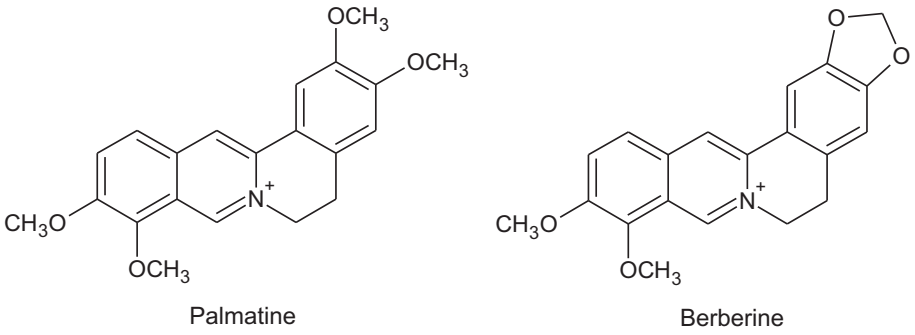

Fig. 1. Chemical structures of palmatine and berberine.

Rodriguez-Antona, 2005). All three CYP1 enzymes are also involved in metabolic activation of various procarcinogens and their induction may hence represent a severe risk for human health (Shimada and Fujii-Kuriyama, 2004). The structural similarity between palmatine and berberine inspired us to investigate whether palmatine affects the expression of CYP1A enzymes in primary cultures of human hepatocytes and human hepatoma HepG2 cells grown as monolayer or spheroids. We found that palmatine activates the AhR and increases mRNA and activity levels of CYP1A1 in HepG2 monolayer, while CYP1A-inducing activity is irrelevant in HepG2 spheroids and primary human hepatocytes.

2. Materials and methods

2.1. Chemicals

Palmatine chloride (No. 361615), berberine chloride (No. B3251) and dimethyl sulfoxide (DMSO) were obtained from Sigma–Aldrich (St. Louis, MO, USA). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) was obtained from Ultra Scientific (North Kingstown, RI, USA). Stock solutions of TCDD, palmatine and berberine in DMSO were stored at −20 °C.

2.2. HepG2 cell monolayer culture and treatment

The human hepatocyte carcinoma HepG2 cell line (No. 85011430, ECACC, Salisbury, UK) was cultured at 37 °C in Dulbecco's modified Eagle's medium (No. D5796, Sigma) supplemented with 1% non-essential amino acids, 100 U/mL penicillin, 100 μg/mL streptomycin (Invitrogen, Carlsbad, CA, USA) and 10% fetal bovine serum (Biochrom, Berlin, Germany) in a humidified atmosphere containing 5% CO_2. The cells were sub-cultured before confluence. For all experiments on monolayer cultures, with the exception of gene reporter assays, HepG2 cells at passage 4–15 were seeded in the complete culture medium at a density of 1×10^5 cells/cm^2. After overnight stabilization, cells were treated in fresh medium with palmatine, berberine or 5 nM TCDD (positive control). Negative controls were treated with 0.1% (*v*/*v*) DMSO only.

2.3. Primary cultures of human hepatocytes

Human tissue samples were obtained from multi-organ donors according to the protocols approved by the local ethics committee of the University Hospital, Olomouc, Czech Republic. Hepatocytes were isolated as described previously (Pichard et al., 1990) and seeded on collagen-coated culture dishes at a density of 1.3×10^5 cells/cm^2 in culture medium (Isom et al., 1985) containing 5% bovine serum (Invitrogen). On the following day, the medium was exchanged for serum-free medium. After 24 h of stabilization, hepatocytes were treated in fresh serum-free medium with 0.1% (*v*/*v*) DMSO (control), palmatine or 5 nM TCDD (positive control). Hepatocyte cultures used in this study were prepared from liver samples of four donors: a 46-year-old man (culture LH45), a 65-year-old man (culture LH47), a 17-year-old woman (culture LH48), and a 38-year-old man (culture LH49).

2.4. Cell viability assay

After treatment of HepG2 cells and hepatocytes with 0.1% DMSO (control), palmatine or 1.5% (*v*/*v*) Triton X-100 (positive control), the cell viability was determined using an MTT reduction assay. In brief, cells were washed with PBS and incubated for 2 h at 37 °C in fresh culture medium containing 0.5 mg/ml MTT (Sigma). After this, culture medium was removed and intracellular formazan produced by active mitochondria was solubilized in DMSO containing 1% ammonia. The absorbance at 540 nm was measured on a spectrophotometric plate reader and used for calculation of relative cell viability where cells treated with DMSO only represented 100% viability.

2.5. Gene reporter assays

A *p1A1-luc* plasmid containing 5′-flanking region (−1566 to +73) of human *CYP1A1* gene subcloned into the *Kpn*I-*Hin*dIII double digested *pGL3-Basic* vector (Promega, Madison, WI, USA) upstream of the firefly luciferase reporter gene was originally prepared by Dr. R. Barouki (Morel and Barouki, 1998). A *pDRE-luc* plasmid containing two inverted repeats of the xenobiotic responsive element of mouse *cyp1a1* upstream of the thymidine kinase promoter and luciferase reporter gene was a kind gift from Dr. L. Poellinger (Karolinska Institute, Stockholm, Sweden). For gene reporter assays, sub-confluent HepG2 cells were detached by trypsinization and cell suspensions in serum-free medium were transiently transfected using jetPEI transfection reagent (Polyplus Transfection, Illkirch, France) with 210 ng of *p1A1-luc* or *pDRE-luc* per 8.3×10^4 cells/well in 24-well plate. Following overnight incubation, cells were treated in serum-containing medium with 0.1% DMSO (control), palmatine or 5 nM TCDD (positive control). After treatment, cell extracts were prepared and analyzed using the Luciferase Assay System (Promega) on an FB12 luminometer (Berthold Detection Systems, Pforzheim, Germany). The luminescence values were used for calculation of fold changes versus control.

2.6. Quantitative real-time PCR

After treatment, total RNA was extracted using the TRI Reagent Solution (Applied Biosystems, Foster City, CA, USA) and the concentration of RNA was determined by spectrophotometry at 260 nm. RNA samples (2 μg) were reverse transcribed using the