Pharmacological Research 120 (2017) 23–33

Contents lists available at ScienceDirect

Pharmacological Research

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

Differential activation of pregnane X receptor by carnosic acid, carnosol, ursolic acid, and rosmarinic acid

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ARTICLE INFO

Article history:
Received 20 November 2016
Received in revised form 24 February 2017
Accepted 8 March 2017
Available online 10 March 2017

Chemical compounds studied in this article:
Carnosic acid (PubChem CID: 65126)
Carnosol (PubChem CID: 442009)
Ursolic acid (PubChem CID: 64945)
Rosmarinic acid (PubChem CID: 5281792)
Pregnenolone 16α-carbonitrile (PubChem CID: 15032)
Rifampicin (PubChem CID: 5381226)
Ketoconazole (PubChem CID: 456201)

Keywords:
Pregnane X receptor
CYP3A4
UGT1A3
ABCB1
Carnosic acid
Carnosol

ABSTRACT

Pregnane X receptor (PXR) regulates the expression of many genes, including those involved in drug metabolism and transport, and has been linked to various diseases, including inflammatory bowel disease. In the present study, we determined whether carnosic acid and other chemicals in rosemary extract (carnosol, ursolic acid, and rosmarinic acid) are PXR activators. As assessed in dual-luciferase reporter gene assays, carnosic acid, carnosol, and ursolic acid, but not rosmarinic acid, activated human PXR (hPXR) and mouse PXR (mPXR), whereas carnosol and ursolic acid, but not carnosic acid or rosmarinic acid, activated rat PXR (rPXR). Dose-response experiments indicated that carnosic acid, carnosol, and ursolic acid activated hPXR with EC_{50} values of 0.79, 2.22, and 10.77 μM, respectively. Carnosic acid, carnosol, and ursolic acid, but not rosmarinic acid, transactivated the ligand-binding domain of hPXR and recruited steroid receptor coactivator-1 (SRC-1), SRC-2, and SRC-3 to the ligand-binding domain of hPXR. Carnosic acid, carnosol, and ursolic acid, but not rosmarinic acid, increased hPXR target gene expression, as shown by an increase in CYP3A4, UGT1A3, and ABCB1 mRNA expression in LS180 human colon adenocarcinoma cells. Rosmarinic acid did not attenuate the extent of hPXR activation by rifampicin, suggesting it is not an antagonist of hPXR. Overall, carnosic acid, carnosol, and ursolic acid, but not rosmarinic acid, are hPXR agonists, and carnosic acid shows species-dependent activation of hPXR and mPXR, but not rPXR. The findings provide new mechanistic insight on the effects of carnosic acid, carnosol, and ursolic acid on PXR-mediated biological effects.

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1. Introduction

Carnosic acid (phenolic diterpene), carnosol (phenolic diterpene), ursolic acid (pentacyclic triterpenoid), and rosmarinic acid (polyphenol) (Fig. 1) are the major chemical constituents of *Rosmarinus officinalis* (also known as rosemary; a food additive and common herb used in the diet), but they are also present in other natural products and health supplements. Carnosol is an oxidative metabolite of carnosic acid [1]. These chemicals have been investigated for many biological activities. For example, carnosic acid has anti-inflammatory, anti-proliferative, and anti-adipogenic properties. It has been shown to induce weight loss, prevent hepatic steatosis, decrease serum triglycerides and cholesterol, and improve glucose tolerance in rodents, thereby it has been suggested to have potential for use in non-alcoholic fatty liver disease [2]. Similarly, carnosol has been reported to have anti-adipogenic [3], chemopreventive [4], and anti-inflammatory properties [4]. Ursolic acid has been most extensively studied for its chemopreventive properties, as shown by numerous *in vitro* and *in vivo* studies [5], although it also has other biological effects, such as anti-inflammatory and anti-obesity effects [6]. Rosmarinic acid has been reported to have anti-oxidant, anti-inflammatory, chemopreventive, and neuroprotective properties [7]. Taken together, these chemicals share several biological activities and have promising therapeutic potential.

Pregnane X receptor (PXR; NR1I2) is a nuclear hormone receptor expressed in many tissues, but predominantly in the liver, small intestine, and colon [8]. It regulates the expression of many drug-metabolizing enzymes involved in the metabolism of endogenous

Abbreviations: DMSO, dimethyl sulfoxide; hPXR, human pregnane X receptor; hRXRα, human retinoid X receptor alpha; hSRC-1, human steroid receptor coactivator-1; hSRC-2, human steroid receptor coactivator-2; hSRC-3, human steroid receptor coactivator-3; mPXR, mouse pregnane X receptor; PCN, pregnenolone 16α-carbonitrile; PXR, pregnane X receptor; rPXR, rat pregnane X receptor; RXRα, retinoid X receptor alpha; SRC, steroid receptor coactivator.

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http://dx.doi.org/10.1016/j.phrs.2017.03.007
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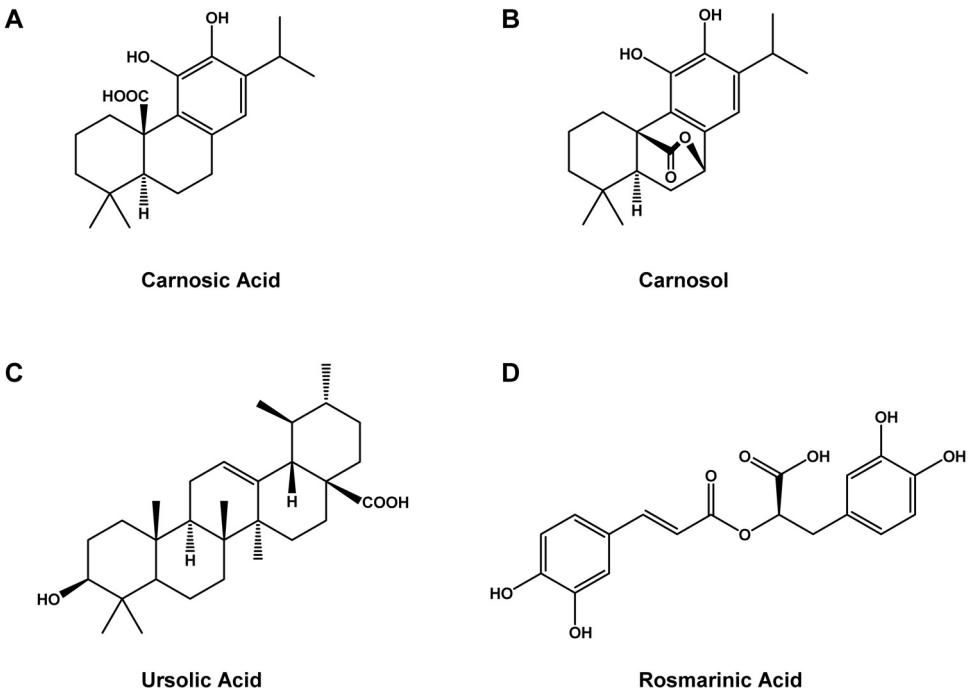

Fig. 1. Chemical structure of (A) carnosic acid, (B) carnosol, (C) ursolic acid, and (D) rosmarinic acid.

and exogenous chemicals, thereby playing a role in drug/chemical-induced toxicity and drug-drug interactions [9]. Other than its role in drug/chemical metabolism, PXR has various physiological and pathophysiological functions, such as lipid metabolism [10] and cell proliferation [11]. PXR has emerged as a therapeutic target for inflammatory bowel disease [12,13].

Previously, carnosic acid (10 μM) was reported to induce the mRNA expression of several drug-metabolizing enzymes, such as CYP3A4 (~5.6–33.2 fold), CYP2B6 (~3.6–4.5 fold), SULT2A1 (~2.4 fold), and UGT1A1 (~2 fold) in primary cultures of human hepatocytes [14]; therefore, it may result in interactions with other drugs metabolized by the same enzymes. However, it is not known how carnosic acid induces these enzymes and whether carnosol, ursolic acid, and rosmarinic acid also induce the expression of some of these enzymes. Given that the expression of CYP3A4 [8], CYP2B6 [15,16], and UGT1A1 [17] are regulated predominantly or in part by PXR, we hypothesized that carnosic acid activates PXR. Therefore, in the present study, we determined the effect of carnosic acid on human PXR (hPXR), mouse PXR (mPXR), and rat PXR (rPXR) transcriptional activity, and compared with that of carnosol, ursolic acid, and rosmarinic acid. We also investigated whether the chemicals induce the expression of hPXR target genes (*CYP3A4*, *UGT1A3,* and *ABCB1)* in hPXR-expressing human colon adenocarcinoma cells. Our findings provide a mechanistic explanation for the induction of various drug-metabolizing enzymes by carnosic acid, and provide new insight into potential drug-chemical interactions and the effects of carnosic acid, carnosol, and ursolic acid on other PXR-mediated biological effects (e.g. inflammatory bowel disease).

2. Materials and methods

2.1. Chemicals and reagents

Carnosic acid, carnosol, ursolic acid, rosmarinic acid, pregnenolone 16α-carbonitrile (PCN), rifampicin, ketoconazole, dextran, Triton X-100, dimethyl sulfoxide (DMSO), Tris-EDTA buffer solution (pH 8.0), trypan blue solution (0.4%), testosterone, prednisolone, and Hanks' balanced salt solution were purchased from Sigma-Aldrich Corporation (St. Louis, MO, U.S.A.) via Sigma-Aldrich Pte. Ltd., Singapore. 6β-Hydroxytestosterone was purchased from Cayman Chemical Company, Ann Arbor, MI, U.S.A. Cytotoxicity Detection Kit [lactate dehydrogenase (LDH)] was bought from Roche Diagnostics Asia Pacific Pte. Ltd., Singapore. Minimum Essential Medium/Earle's Balanced Salts (MEM/EBSS) culture medium (#SH30244.01), fetal bovine serum (#SV30160.03), heat-inactivated charcoal/dextran-treated fetal bovine serum (#SH30068.03HI), MEM non-essential amino acids (100×), trypsin-EDTA (0.25%), and phosphate buffered saline (pH 7.4) were of HyClone™ brand purchased from GE Healthcare Life Sciences (Buckinghamshire, U.K.), while L-glutamine (200 mM) was from PAA Laboratories GmbH (Cölbe, Germany; part of GE Healthcare Life Sciences) and penicillin G-streptomycin (100×) was from PAN-Biotech GmbH (Aidenbach, Germany). Opti-MEM I Reduced Serum Medium (Gibco™), UltraPure 0.5 M EDTA pH 8.0 (Invitrogen™), and UltraPure DNase/RNase-Free Distilled Water (Invitrogen™) were purchased from Thermo Fisher Scientific, Inc. (Waltham, MA, U.S.A.). FuGENE 6 transfection reagent, Dual-Luciferase Reporter Assay System, ReliaPrep RNA Cell Miniprep, GoScript Reverse Transcription System, QuantiFluor dsDNA System, GoTaq qPCR Master Mix, and Wizard SV Gel and PCR Clean-up System were purchased from Promega Corporation (Madison, WI, U.S.A.). All other commercially available chemicals were of analytical or molecular biology grade.

2.2. Plasmids

pCMV6-XL4-hPXR, pCMV6-entry-mPXR, pCMV6-AC-rPXR, pCMV6-XL4, pCMV6-entry, and pCMV6-AC were purchased from OriGene Technologies, Inc. (Rockville, MD, U.S.A.). PathDetect pFR-luc *trans*-reporter (contains five tandem repeats of yeast GAL4 binding sites) was bought from Agilent Technologies (Santa Clara, CA, U.S.A.). The pVP16 empty vector and pM empty vector (contains the yeast GAL4 DNA-binding domain) were obtained from the Matchmaker Mammalian Two-Hybrid Assay Kit (Takara Bio USA, Inc., Mountain View, CA, U.S.A.). *Renilla reniformis* luciferase

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