Pharmacological Research 100 (2015) 64–72

Contents lists available at ScienceDirect

Pharmacological Research

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

3-Hydroxyflavone and structural analogues differentially activate pregnane X receptor: Implication for inflammatory bowel disease

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ARTICLE INFO

Article history:
Received 13 July 2015
Received in revised form 29 July 2015
Accepted 29 July 2015
Available online 31 July 2015

Chemical compounds studied in this article:
3-Hydroxyflavone (PubChem CID: 11349)
Galangin (PubChem CID: 5281616)
Datiscetin (PubChem CID: 5281610)
Kaempferol (PubChem CID: 5280863)
Morin (PubChem CID: 5281670)
Quercetin dihydrate (PubChem CID: 5284452)
Isorhamnetin (PubChem CID: 5281654)
Tamarixetin (PubChem CID: 5281699)
Myricetin (PubChem CID: 5281672)
Syringetin (PubChem CID: 5281953)

Keywords:
Pregnane X receptor
CYP3A4
CYP3A5
ABCB1
Flavonol
3-Hydroxyflavone

ABSTRACT

Pregnane X receptor (PXR; NR1I2) is a member of the superfamily of nuclear receptors that regulates the expression of genes involved in various biological processes, including drug transport and biotransformation. In the present study, we investigated the effect of 3-hydroxyflavone and its structurally-related analogues on PXR activity. 3-Hydroxyflavone, galangin, kaempferol, querceetin, isorhamnetin, and tamarixetin, but not but not datiscetin, morin, myricetin, or syringetin, activated mouse PXR, as assessed in a cell-based reporter gene assay. By comparison, 3-hydroxyflavone activated rat PXR, whereas 3-hydroxyflavone, galangin, quercetin, isorhamnetin, and tamarixetin activated human PXR (hPXR). A time-resolved fluorescence resonance energy transfer competitive ligand-binding assay showed binding to the ligand-binding domain of hPXR by 3-hydroxyflavone, galangin, quercetin, isorhamnetin, and tamarixetin. 3-Hydroxyflavone and galangin, but not quercetin, isorhamnetin, or tamarixetin, recruited steroid receptor coactivator (SRC)-1, SRC-2, and SRC-3 to hPXR. In LS180 human colon adenocarcinoma cells, 3-hydroxyflavone, quercetin, and tamarixetin increased CYP3A4, CYP3A5, and ABCB1 mRNA expression, whereas galangin and isorhamnetin increased CYP3A4 and ABCB1 but not CYP3A5 mRNA expression. Datiscetin, kaempferol, morin, myricetin, and syringetin did not attenuate the extent of hPXR activation by rifampicin, suggesting they are not hPXR antagonists. Overall, flavonols activate PXR in an analogue-specific and species-dependent manner. Substitution at the C2′ or C5′ position of 3-hydroxyflavone with a hydroxyl or methoxy group rendered it incapable of activating hPXR. Understanding the structure-activity relationship of flavonols in hPXR activation may facilitate nutraceutical development efforts in the treatment of PXR-associated intestinal diseases, such as inflammatory bowel disease.

1. Introduction

Pregnane X receptor (PXR; NR1I2) is a member of the superfamily of nuclear receptors [1]. It is expressed in various tissues, including liver, duodenum, jejunum, ileum, and colon [2]. Examples of genes regulated by PXR include those function in drug biotransformation, such as *CYP3A4* [3–5] and *CYP3A5* [6], and in drug transport, such as *ABCB1* (P-glycoprotein) [7,8]. PXR activation may confer therapeutic benefits in certain intestinal diseases, including inflammatory bowel disease [9–12]. For example, activation of PXR by rifaximin has been reported to ameliorate inflammatory bowel diseases [9–11] and induce remission in patients with moderately active Crohn's disease [13] or irritable bowel syndrome [14]. However, rifaximin may cause adverse effects, such as hepatic steatosis [15], toxic epidermal necrolysis [16], and diarrhea

Abbreviations: CAS, chemical abstracts service; 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; PCN, pregnenolone 16α-carbonitrile; PXR, pregnane X receptor; RXRα, retinoid X receptor alpha; SR12813, tetraethyl 2-(3,5-di-tert-butyl-4-hydroxyphenyl)ethenyl-1,1-bisphosphonate; SRC, steroid receptor coactivator; TR-FRET, time-resolved fluorescence resonance energy transfer.

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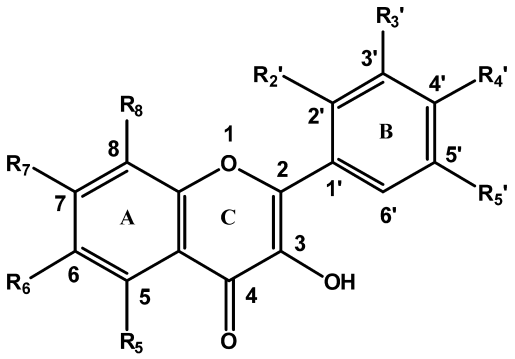
http://dx.doi.org/10.1016/j.phrs.2015.07.031
1043-6618/



Flavonols	Ring A				Ring B			
	R_5	R_6	R_7	R_8	$R_{2'}$	$R_{3'}$	$R_{4'}$	$R_{5'}$
3-Hydroxyflavone	H	H	H	H	H	H	H	H
Galangin	OH	H	OH	H	H	H	H	H
Datiscetin	OH	H	OH	H	**OH**	H	H	H
Kaempferol	OH	H	OH	H	H	H	**OH**	H
Morin	OH	H	OH	H	**OH**	H	**OH**	H
Quercetin	OH	H	OH	H	H	**OH**	**OH**	H
Isorhamnetin	OH	H	OH	H	H	**OCH_3**	**OH**	H
Tamarixetin	OH	H	OH	H	H	**OH**	**OCH_3**	H
Myricetin	OH	H	OH	H	H	**OH**	**OH**	**OH**
Syringetin	OH	H	OH	H	H	**OCH_3**	**OH**	**OCH_3**

Fig. 1. Chemical structure of 3-hydroxyflavone and structural analogues.

[14]. Therefore, there is a need to discover alternative locally-acting and effective PXR agonists that can be developed for the treatment of PXR-associated intestinal diseases, such as inflammatory bowel disease.

Flavonoids are present in plants, food, herbal medicines, and health supplements [17]. They represent a class of polyphenolic chemicals with a three-ring structure; i.e., a heterocyclic ring and two aromatic rings [18]. They can be divided into various subclasses based on the type of substituent in the heterocyclic ring. The subclasses are flavonol, flavone, flavanol, flavanone, flavanolol, and isoflavone. As examples of chemicals in these subclasses, quercetin and kaempferol are flavonols, apigenin and chrysin are flavones, hesperidin and naringenin are flavanones, epicatechin and gallocatechin are flavanols (also called catechins), taxifolin and hydrorobinetin are flavanonols, and genistein and daidzein are isoflavones [18]. Shown in Fig. 1 are the chemical structures of the flavonol scaffold (also known as 3-hydroxyflavone) and several of its analogues, including quercetin, galangin, datiscetin, kaempferol, morin, isorhamnetin, tamarixetin, myricetin, and syringetin. Previous cell culture studies reported hPXR activation by quercetin [19], kaempferol [19], tamarixetin [19], and isorhamnetin [20]. However, conflicting data exist for quercetin and kaempferol [19,21–26]. In a recent mouse study, isorhamnetin was shown to activate PXR and compromise the NF-κB signaling pathway, thereby ameliorating chemically-induced colitis [20].

Given that PXR is a potential therapeutic target for certain intestinal diseases [9], it is important to investigate the structure-activity relationship of flavonols in PXR activation as a way to provide a basis for future nutraceutical development efforts in identifying novel therapy for these diseases. Therefore, the objective of the present study was to investigate the effect of 3-hydroxyflavone and several of its structural analogues (galangin, datiscetin, kaempferol, morin, quercetin, isorhamnetin, tamarixetin, myricetin, and syringetin) on the activity of hPXR and compare to the activity of mouse PXR (mPXR) and rat PXR (rPXR). Experiments were also performed to determine whether these chemicals bind to the ligand-binding domain of hPXR, recruit coactivators the receptor, and increase the expression of hPXR target genes. The results are discussed in the context of the analogue-specific and species-dependent activation of PXR by flavonols and their mode of receptor activation.

2. Materials and methods

2.1. Chemicals and reagents

The following flavonols were purchased from Indofine Chemical Company, Inc. (Hillsborough, NJ, U.S.A.): 3-hydroxyflavone (CAS #577-85-5), galangin (3,5,7-trihydroxyflavone; CAS #548-83-4), datiscetin (3,5,7,2′-tetrahydroxyflavone; CAS #480-15-9), kaempferol (3,5,7,4′-tetrahydroxyflavone; CAS #520-18-3), morin (3,5,7,2′,4′-pentahydroxyflavone; CAS #480-16-0), isorhamnetin (3,5,7,4′-tetrahydroxy-3′-methoxyflavone; CAS #480-19-3), tamarixetin (4′-methoxy-3,5,7,3′-tetrahydroxyflavone; CAS #603-61-2), myricetin (3,5,7,3′,4′,5′-hexahydroxyflavone; CAS #529-44-2), and syringetin (3′,5′-dimethoxy-3,5,7,4′-tetrahydroxyflavone; CAS #4423-37-4). Quercetin dihydrate (3,5,7,3′,4′-pentahydroxyflavone; CAS #6151-25-3), rifampicin (CAS #13292-46-1), and pregnenolone 16α-carbonitrile (PCN; CAS #1434-54-4), and sodium phenobarbital (CAS #50-06-6) were purchased from Sigma-Aldrich (St Louis, MO, U.S.A.). Tetraethyl 2-(3,5-di-*tert*-butyl-4-hydroxyphenyl) ethenyl-1,1-bisphosphonate (SR12813; CAS #126411-39-0) was obtained from Enzo Life Sciences, Inc. (Plymouth Meeting, PA, U.S.A.) and ketoconazole (CAS #65277-42-1) was from Toronto Research Chemicals (Toronto, ON, Canada). Charcoal/dextran-treated, heat-inactivated fetal bovine serum (Hyclone Laboratories, Logan, UT, U.S.A.) was bought from Thermo Fisher Scientific, Inc. (Nepean, ON, Canada). Heat-inactivated fetal bovine serum (untreated; Gibco), Opti-MEM, PureLink RNA Mini Kit, SuperScript III reverse transcriptase, PicoGreen double-stranded DNA Quantitation Kit, Platinum *Taq* DNA polymerase, SYBR Green I solution, and all other cell culture and PCR reagents were obtained from Life Technologies, Inc. (Carlsbad, CA, U.S.A.). FuGENE 6 transfection reagent and Dual-Luciferase Reporter Assay System were purchased from Promega (Madison, WI, U.S.A.).

2.2. Plasmids

pCMV6-entry-mPXR, pCMV6-entry-rPXR, pCMV6-XL4-hPXR, pCMV6-entry, and pCMV6-XL4 were purchased from OriGene Technologies, Inc. (Rockville, MD, U.S.A.). The other plasmids used in the present study have been described elsewhere [27,28].

2.3. Cell culture

HepG2 human hepatocellular carcinoma cells and LS180 human colon adenocarcinoma cells were purchased from the American Type Culture Collection (Manassas, VA, U.S.A.) and cultured in Minimum Essential Medium supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% v/v heat-inactivated fetal bovine serum [27].

2.4. Transient transfection, treatment, and reporter gene assays

HepG2 cells were cultured as described previously [29]. At 5 h after plating, cells were transfected with 20 μl of a transfection master mix containing FuGENE 6 transfection reagent (3 μl/μg of DNA), serum-free Opti-MEM (20 μl/well), and various plasmids for 24 h. The plasmids used in the PXR-dependent reporter gene assay have been described elsewhere [27,28].