Modulation of PXR activity by ginger and its constituents

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OBJECTIVE :To study the PXR-mediated herb-drug interaction potential of ginger to assure the safety of ginger-based dietary supplement products

Abstract

Ginger root (*Zingiber officinale*) is widely used in food as a spice and flavoring agent, and it is an integral part of Southeast Asia's traditional medicine to treat various human ailments such as the common cold, inflammation, rheumatic disorder, and gastrointestinal discomforts. A quick search in NIH's Dietary Supplement Label Database resulted in >3,500 ginger-based products sold via major retailers in the US. In our pursuit to assure the quality and safety of botanical ingredients in various matrices and due to a lack of scientific data on these ingredients' potential adverse effects, a root extract of ginger and its principal components were investigated for possibility of the herb-drug interactions through activating pregnane-X-receptor (PXR), which regulates the expression of drug-metabolizing enzymes and transporters (DMET). Our preliminary findings suggested that ethanolic extract of ginger at a 20 µg/mL concentration activated PXR up to 2.31-fold in LS-174T cells. Among the constituents, 6-paradol, 6-shagoal, and dehydro-6-gingerdione at 30 µM activated the PXR up to 2.1, 3.4, and 2.9-fold, respectively. However, 6-gingerol and 6-gingerdiol were less potent, and fold activation was below two-fold. Gene expression analysis by RT-PCR showed increased expression of CYP3A4, CYP2C9, CYP1A2, CYP2B6, and P-gP mRNA by the extract, 6-paradol, 6-shagoal, and dehydro-6-gingerdione to variable extents.

Introduction

- Ginger is a perennial herb belonging to the family *Zingiberaceae*.
- The recent clinical or preclinical investigations showed that ginger significantly reduces the intensity of pain during primary dysmenorrhea, including functional dyspepsia, reduces obesity, and protects from radiations.

Ginger Rhizome

- Gingerols (6-, 8-, and 10-gingerol), shogaols (6-, 8-, and 10-shogaols), and paradols (6-, 8-,10-paradols) are reported to be responsible for a wide range of pharmacological activities.
- In 2017, ginger ranked 9th among the top 40 selling herbal supplements and showed a 5.2% selling increment since 2016.
- PXR and AhR are the transcription factors known to upregulate the expression of drug-metabolizing enzymes (CYPs) and transporters (P-gp). Orally ingested dietary phytochemicals, including ginger, may act as agonists or antagonists for PXR and AhR resulting in dysregulation of CYPs, alter the metabolism of pharmaceutical drugs resulting in unexpected adverse effects.

Materials and Methods

Cell culture: LS174T (human colon adenocarcinoma cells) were obtained from ATCC, Manassas, USA. Cells were cultured in DMEM/F12 medium supplemented with 10% FBS, 2.4 g/L sodium bicarbonate, 100 µg/mL streptomycin and 100 units/mL penicillin at 37 °C with 95% humidity and 5% CO_2 .

Luciferase reporter gene assay for PXR activation: PXR activation assay was performed in LS174T cells transiently transfected with pSG5-hPXR and PCR-5 plasmid DNA (25 µg each) by electroporation at 170 V, 1 pulse for 70 msec as described earlier [1,2]. Cells (50,000 cells well) were seeded in 96-well plates, and upon confluency, they were treated with test samples for 24 h. Media was removed, and luciferase activity was measured. Fold increase in the luminescence of sample treated cells was calculated compared to vehicle-treated cells as an index of PXR activation.

Quantitative analysis of gene expression

Transfection of cells and isolation of RNA: LS174T cells (transfected as above) were treated with ginger extract and pure compounds for 24 h. Total RNA was isolated using the Quick-start protocol (Qiagen® kit). The quantity of isolated RNA was measured using Bio-Tek, Synergy HT Multi-Mode Microplate reader.

Complementary DNA and real-time (RT) PCR analysis: The validated PCR™ SYBR® Green Assay, forward and reverse primers for CYP3A4 (qHsaCID0012316), CYP2C9 (qHsaCED0044817), CYP1A2 (qHsaCID0015160), CYP2B6 (qHsaCED0038976), and P-gp (qHsaCED0002291) were purchased from Bio-Rad Laboratories, USA.

The first-strand complementary DNA (cDNA) was synthesized using a total of 1 µg of RNA as a template in Bio-Rad iScript™ Reverse Transcription Supermix. This cDNA was used as the template with Bio-Rad, iTaq™ Universal SYBR® Green Supermix. RT-PCR was performed in a 96-well plate by the CFX connect real-time PCR detector system (Bio-Rad) as described previously [2].

Cytochrome P450s (CYP) inhibition assay: CYP inhibition assays for CYP3A4, CYP2C9, CYP2B6, and CY1A2 were performed using Vivid® kits (Invitrogen, Carlsbad, CA). Briefly, stock solutions of extracts, purified compounds, and controls were serially diluted in methanol and incubated with human recombinant cytochrome P450 (BACULOSOMES®), NADP⁺, and regeneration reagents in 96-well round-bottom plates at room temperature for 10 min. The reaction was started by adding 10 µL of 10x specific fluorescent substrate of CYP3A4, CYP2C9, or CYP1A2. After incubation, stop-reagent was added, and fluorescence was measured. The IC_{50} values were calculated from concentration-response curves.

Results and Discussion

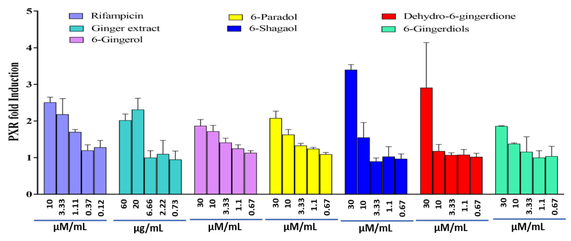
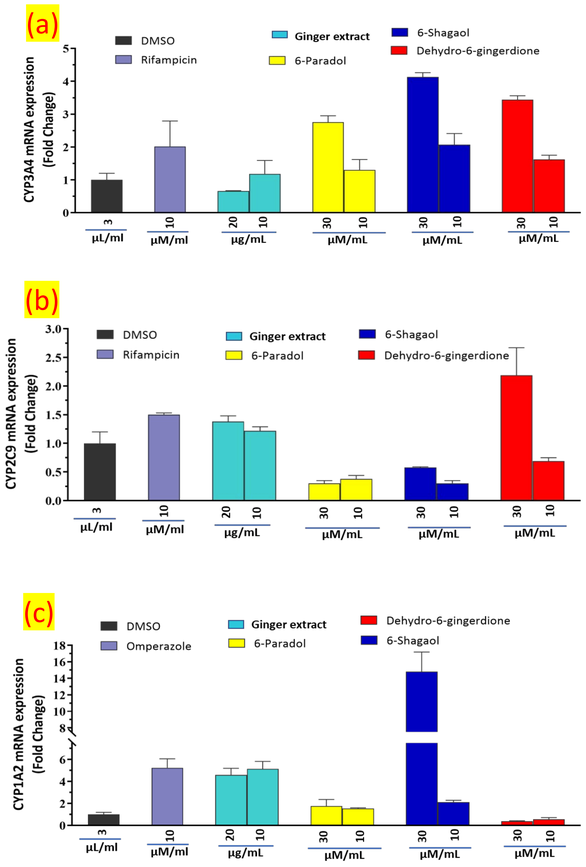


Figure 1. Ginger extract and its pure phytochemicals activate the nuclear transcription factor PXR in intestinal cell line LS174T. Cells were transfected with pSG5-hPXR and PCR-5 plasmid DNA and treated with indicated concentrations of ginger extract, pure phytochemicals and positive control (rifampicin) for 24 h. After incubation, luciferase activity was measured.



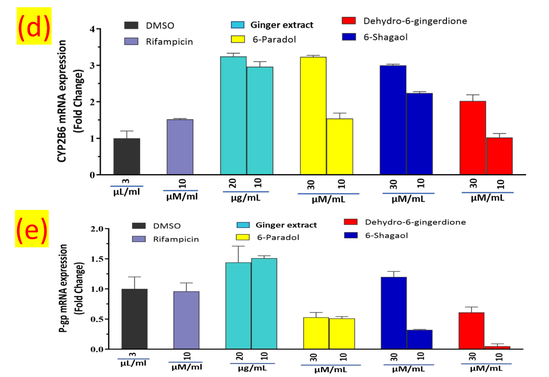


Figure 2. Inductive effects of ginger extracts and its phytochemicals on mRNA of CYP3A4 **(a)**, CYP2C9 **(b)**, CYP1A2 **(c),** CYP2B6 **(d)** and P-gp **(e)**. Transfected LS174T cells were treated with indicated concentrations of extract, pure compounds and positive control. After incubation, RNA was isolated and RT-PCR was performed by a CFX connect real-time PCR detector system.

Table 1. Inhibition of CYP3A4, CYP2C9, and CYP1A2 by ginger extract and its phytochemicals.

Sample	IC_{50} values (µg/mL for extract and µM for pure compounds)		
	CYP3A4	CYP2C9	CYP1A2
Ginger extract	7.15	1.5	8.45
6-Gingerol	NA	5.4	19.5
6-Paradol	13	1.4	4.7
6-Shagoal	8.5	0.8	4.25
Dehydro-6-gingerdione	6.2	3	6.5
6-Gingerdiol	16.5	8.5	>50

NA = Not Achieved

Conclusions

- Extract of ginger and its principal phytoconstituents, namely 6-paradol, 6-shogaol and dehydro-6-gingerdione, activated PXR in a concentration-dependent manner that was reflected in increased expression of CY3A4, CYP1A2 and CYP2B6 mRNA but not in 2C9 and P-gp mRNA .
- Strong inhibition of CYP3A4, 2C9 and 1A2 by ginger extract and its phytochemicals could also play a role in its overall effect on drug metabolism and clearance.
- Clinical studies are warranted to confirm the significance of HDI risk associated with ginger based dietary supplements.

References

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