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Dietary polyacetylenes of the falcarinol type are inhibitors of breast cancer resistance protein (BCRP/ABCG2)

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ABSTRACT

Polyacetylenes of the falcarinol type are present in vegetables such as carrots and parsley. They display interesting bioactivities and hold potential as health-promoting and therapeutic agents. In this study, falcarinol, falcarindiol, falcarindiol 3-acetate and falcarindiol 3,8-diacetate were examined for their modulation on breast cancer resistance protein (BCRP/ABCG2), an efflux transporter important for xenobiotic absorption and disposition, and multidrug resistance in cancer. Falcarinol, falcarindiol, and falcarindiol 3-acetate were extracted from carrots and falcarindiol 3,8-diacetate prepared from falcarindiol. Their modulatory effects on ABCG2 were studied using three methods—mitoxantrone accumulation, vesicular transport, and ATPase assay. The polyacetylenes inhibited mitoxantrone (an ABCG2 substrate) efflux in ABCG2-overexpressing HEK293 cells. The inhibitory effect was confirmed in the vesicular transport assay, in which concentration-dependent inhibition of methotrexate (an ABCG2 substrate) uptake into ABCG2-overexpressing Sf9 membrane vesicles was observed (IC_{50} = 19.7–41.7 μM). The polyacetylenes also inhibited baseline and sulfasalazine-stimulated vanadate-sensitive ATPase activities in ABCG2-overexpressing Sf9 membrane vesicles (IC_{50} = 19.3–79.3 μM). This is the first report of an inhibitory effect of polyacetylenes on ABCG2. These results indicate a prospective use of polyacetylenes as multidrug resistance reversal agents, a possible role of ABCG2 in the absorption and disposition of polyacetylenes, and potential food-drug interactions between polyacetylene-rich foods and ABCG2 substrate drugs.

1. Introduction

Polyacetylenes are a large group of natural compounds primarily produced by higher plants of the *Apiaceae*, *Araliaceae* and *Asteraceae* families (Christensen, 2011). In the human diet, polyacetylenes can be found in common vegetables and herbs of the *Apiaceae* family such as carrots, celery, parsnip, coriander, and parsley (Christensen, 2011). Similar to other plant-derived secondary metabolites, or phytochemicals, the polyacetylenes demonstrate a broad range of bioactivities and are believed to contribute to the health benefits associated with the consumption of fruit and vegetables (Crozier et al., 2009; Liu, 2003). In particular, aliphatic C_{17}-polyacetylenes of the falcarinol type have been shown to exhibit potent antimicrobial, anti-inflammatory, and anticancer effects (Christensen, 2011; Christensen and Brandt, 2006). In carrots (*Daucus carota* L.), more than a dozen structurally distinct C_{17}-polyacetylenes have been identified and quantified in the range of 74 – 4846 μg/g dry weight in different carrot varieties (Killeen et al., 2013). Among these, falcarinol, falcarindiol and falcarindiol 3-acetate (Fig. 1) consistently occur at the highest levels (Killeen et al., 2013; Schmiech et al., 2009). Falcarindiol 3,8-diacetate (Fig. 1), on the other hand, has not been shown to occur naturally but has previously been prepared by acetylation of falcarindiol (Muir et al., 1982; Park and Kim, 1995).

Breast cancer resistance protein (BCRP/ABCG2) is a member of the evolutionarily-conserved superfamily of ATP (adenosine triphosphate)-binding cassette (ABC) transporters that have a characteristic role in coupling ATP hydrolysis to the cross-membrane translocation of a variety of substances (Dean and Annilo, 2005). ABCG2, like its close relatives *P*-glycoprotein (*P*-gp/ABCB1) and multidrug resistance

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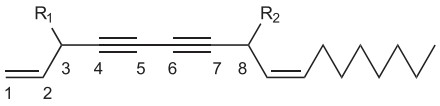

Polyacetylene	R_1	R_2
Falcarinol	OH	H
Falcarindiol	OH	OH
Falcarindiol 3-acetate	OAc	OH
Falcarindiol 3,8-diacetate	OAc	OAc

Fig. 1. Chemical structures of polyacetylenes used in the present study.

protein 2 (MRP2/ABCC2), is primarily involved in the apical efflux of various xenobiotics including many clinically relevant drugs, although it is also known for facilitating the passage of endogenous substrates such as vitamins (B2, B9, and K3) and uric acid (Chen et al., 2003; Shukla et al., 2007; van Herwaarden et al., 2007; Woodward et al., 2009). Predominantly expressed at the apical membrane of epithelial cells of organs such as the gastrointestinal tract, liver, and kidney, as well as physiological barriers such as the blood–brain, blood–testis, and maternal-fetal barriers (Fetsch et al., 2006; Maliepaard et al., 2001), ABCG2 has been implicated in regulating the absorption and distribution of substrate xenobiotics into the systemic circulation and target organs (Schinkel and Jonker, 2012). Accordingly, ABCG2 is increasingly recognized as an important transporter involved in the Phase III efflux of xenobiotics (Szakacs et al., 2008). In addition, overexpression of ABCG2 has also been attributed as one of the prominent mechanisms for malignant tissues to evade exposure to chemotherapeutic drugs, leading to multidrug resistance in many human cancers (Natarajan et al., 2012).

ABCG2 is also known to interact with various dietary phytochemicals and their metabolites. Phytochemicals such as various flavonoids and stilbenes have been demonstrated to inhibit ABCG2-mediated transport (Cooray et al., 2004; Dreiseitel et al., 2009; Tan et al., 2013; Valdameri et al., 2012; Zhang et al., 2004). A few phytochemicals and/or their Phase II conjugates have also been shown to be substrates of ABCG2 in vitro (An et al., 2011; An and Morris, 2011; Breedveld et al., 2007; van de Wetering et al., 2009). Moreover, animal studies utilizing *Abcg2* knock-out mice have highlighted the role of ABCG2 in modulating the bioavailability of a number of dietary phytochemicals and their metabolites (Enokizono et al., 2007; van de Wetering et al., 2009). In the present study, we provide evidence that falcarinol, falcarindiol, falcarindiol 3-acetate, and falcarindiol 3,8-diacetate exhibit a distinct affinity for ABCG2.

2. Materials and methods

2.1. Materials

Mitoxantrone, methotrexate, and sulfasalazine were procured from Sigma-Aldrich (Auckland, NZ). Ko143 was obtained from Tocris Biosciences (Bristol, UK). [^{3}H]methotrexate was purchased from American Radiolabeled Chemicals (MO, US).

2.2. Isolation of polyacetylenes

Fresh commercial baby carrots (540 g) were diced and lyophilized. The resulting 57.7 g of dry tissue was extracted into 600 ml of dichloromethane with gentle agitation. The extract was filtered and the carrot matter was extracted with a further 600 ml of dichloromethane. The resulting extracts were relieved of solvent in vacuo and combined, yielding 750 mg of extract. This extract was fractionated on a silica gel (Sigma-Aldrich, 35–75 μm) column (10 g) using a dichloromethane/ethyl acetate gradient elution. Falcarinol, falcarindiol 3-acetate, and falcarindiol eluted in 100% dichloromethane, 90% dichloromethane and 80% dichloromethane, respectively. Compounds were identified by gas chromatography–mass spectrometry and [^{1}H] nuclear magnetic resonance (NMR) with data corresponding to those previously published (Czepa and Hofmann, 2004; Metzger and Barnes, 2009). The crude polyacetylenes were purified by semi-preparative high-performance liquid chromatography (HPLC) using an Agilent 1260 Infinity and Phenomenex Luna (5 μm) C18 column (10 × 250 mm). Gradient elution from 70 to 88% methanol in water was applied over 30 min, followed by 100% methanol for 5 min and a 5 min re-equilibration. Falcarindiol, falcarindiol 3-acetate and falcarinol eluted at 10.6, 12.6 and 17.0 min, respectively. The flow rate was 4.5 ml/min with an injection volume of 200 μl and UV detection at 210 nm. Falcarinol, falcarindiol and falcarindiol 3-acetate were isolated at purities of 99 and 95%, respectively, as determined by gas chromatography with flame ionization detection as previously described (Killeen et al., 2013).

2.3. Preparation of falcarindiol 3,8-diacetate

Falcarindiol 3,8-diacetate was investigated in this study to examine if it exhibits a different activity profile from the other three natural polyacetylenes. Falcarindiol (42 μmol) was isolated as described above and dissolved in dried chloroform (500 μl). Acetic anhydride (90 μmol) and dried pyridine (90 μmol) were added and the reaction was allowed to proceed overnight at room temperature in the dark. After 18 h, the reaction was stopped with the addition of 200 μl of distilled water. The organic layer was separated and the aqueous layer was washed with ethyl acetate (3 × 5 ml). The organic phases were combined, dried over magnesium sulphate and liberated of solvent in vacuo affording falcarindiol 3,8-diacetate (crude). The crude product was dissolved in methanol and purified by semi-preparative HPLCas described above (retention time of 16.2 min) and is fully characterized here for the first time (19 μmol; 45% yield). Falcarindiol 3,8-diacetate (Fig. 1). Colorless gum (6.5 mg); $[\alpha]_D^{25}+87.8$ (c 0.1, methanol); UV/vis (methanol): λ_{max} (log ε)=285 (1.0), 270 (1.0), 260 (0.9), 250 (0.7); electrospray ionization mass spectrometry: *m/z* (367.1857 $[M+Na]^+$ $C_{21}H_{28}O_4$); [^{1}H] NMR (500 MHz, deuterated chloroform ($CDCl_3$); assigned by correlation spectroscopy (COSY)): δ 5.36 [^{1}H, dd, J=10.0, 1.1 Hz, H-C_{cis}(1)], 5.55 [^{1}H, dd, J=16.8, 1.2 Hz, H-C_{trans}(1)], 5.87 [^{1}H, ddd, 16.8, 9.9, 5.8 Hz, H-C(2)], 5.91 [^{1}H, dd, J=5.7, 0.8 Hz, H-C(3)], 6.14 [^{1}H, brd, J=8.8 Hz, H-C(8)], 5.48 [^{1}H, ddt, 10.5, 8.9, 1.6 Hz, H-C(9)], 5.68 [^{1}H, dtd, 10.7, 7.6, 1.1 Hz, H-C(10)], 2.15 [^{2}H, brq, 7.1 Hz, H-C(11)], 1.39 [^{2}H, brt, 5.9 Hz, H-C(12)], 1.20–1.34 [^{8}H, m, H-C(13–16)], 0.89 [^{3}H, t, 7.1 Hz, H-C(17)], 2.09 [^{3}H, s, H-Ac(3)], 2.11 [^{3}H, s, H-Ac(8)]; [^{13}C] NMR (125 MHz, $CDCl_3$; HMQC, HMBC): δ 119.76 [C-(1)], 136.50 [C-(2)], 64.35 [C-(3)], 75.00 [C-(4)], 70.70 [C-(5)], 69.08 [C-(6)], 76.67 [C-(7)], 76.67 [C-(8)], 123.58 [C-(9)], 131.81 [C-(10)], 27.85 [C-(11)], 29.11 [C-(12)], [29.10 C-(13)], 29.08 [C-(14)], 31.77 [C-(15)], 22.61 C-[(16)],14.08 [C-(17)], 169.39 [Acetate(18)], 20.89 [Acetate(19)], 169.42 [Acetate(20)], 20.83 [C-(21)].

2.4. Cell culture

Wild-type and ABCG2-overexpressing HEK293 (human embryonic kidney) cell lines (HEK293/WT and HEK293/ABCG2) were generously provided by Prof. P. Borst (Netherlands Cancer Institute, the Netherlands) (de Wolf et al., 2008). Cells were cultured in Dulbecco's modified Eagle's medium (Cat. No. 11995-065, Life Technologies, Auckland, NZ) supplemented with 10% fetal bovine serum in a humidified incubator maintaining 5% CO_2 at 37 °C. HEK293/ABCG2 cells were grown in the presence of 200 μg/ml of G418 (Sigma-Aldrich, Auckland, NZ). Sf9 insect cells were cultured in SF-900 III medium (Life Technologies, Auckland, NZ) supplemented with 10% fetal bovine serum. Cells were maintained at 28 °C on an orbital