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Prunin- and hesperetin glucoside-alkyl (C_4–C_{18}) esters interaction with Jurkat cells plasma membrane: Consequences on membrane physical properties and antioxidant capacity

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ABSTRACT

Prunin (P)- and hesperetin glucoside (HG)-alkyl esters are lipid-soluble compounds with antimicrobial and antioxidant capacities in vitro. The effects of P- and HG-alkyl (C_4–C_{18}) esters (0.1–100 μM) on human leukemia T (Jurkat) cells viability and plasma membrane fluidity were evaluated. After 1 h of exposure, cell viability was not affected in the range 0.1–10 μM. The decrease of cell viability found at 100 μM concentration depended on the length of the alkyl chain and reached a maximum with C_6–C_{12} derivatives. At this concentration, cell hyperpolarization and shrinkage were also observed. Cell plasma membrane fluidity was not affected, regardless the depths of the membrane level evaluated, but mild changes in plasma membrane hydration were found. Esterification did not affect the antioxidant capacity of P and HG (0.1–10 μM) against 1 mM H_2O_2. When exposed to 1 mM AAPH, P-alkyl esters retained P antioxidant capacity, but HG-derivatives acted as pro-oxidants. Together, present experimental evidences suggest that short term exposures to 0.1–10 μM concentrations of P- and HG-alkyl (C_4–C_{18}) esters can be considered safe for cultured human cells, and further studies are required to investigate their long term effects, as well their safety for human consumption.

1. Introduction

Flavanone glycosides constitute a subclass of flavonoids present in *Citrus* fruits. The growing knowledge of their beneficial effects on human health (Benavente-Garcia and Castillo, 2008; Itoh et al., 2009; Gonzalez-Molina et al., 2010; Selvaraj and Pugalendi, 2010; Tsao, 2010; Al-Ashaal and El-Sheltawy, 2011) makes these compounds and their derivatives good candidates to be used in the pharmaceutical and food industries (for a review see Garg et al., 2001).

Aglicons naringenin (4′,5,7-trihydroxyflavanone) and hesperetin (4′-methoxy-3′,5,7-trihydroxyflavanone) (Fig. 1A) can bear a glucose moiety bound to the hydroxyl group in position 7 of the A ring, rendering the glucosyl derivatives named naringenin 7-*O*-glucoside or prunin (P) and hesperetin 7-*O*-glucoside (HG), respectively (Fig. 1B). While P has a hydroxyl group in 4′ of ring B, HG has a hydroxyl group in 3′ and a methoxyl group in 4′ (Fig. 1B). Further chemical modifications of these flavanones can be achieved in vitro. Recently, an enzymatic method that allows the esterification of the hydroxyl group in position 6 of the glucoside moiety in P with alkyl vinyl esters or alkyl acids of different chain length was described (Fig. 1C) (Céliz and Daz, 2011). Using the same methodology, a series of alkyl esters of HG were synthesized (Fig. 1C). All

Abbreviations: 12-AS, 12-(9-anthroyloxy) stearic acid; 16-AP, 16-(9-anthroyloxy) palmitic acid; 6-AS, 6-(9-anthroyloxy) stearic acid; AAPH, 2,2′-azobis-2-methyl-propanimidamide, dihydrochloride; BA, butyric acid; C_{11}-BODIPY, 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid; DA, decanoic acid; DCDCDHF, 5-(and-6)-carboxy-2′,7′-dichloro-dihydrofluorescein-diacetate; DCF, dichlorofluorescein; $DiBaC_4(3)$, bis-(1,3-dibutylbarbituric acid)trimethine oxanol; DMSO, dimethylsulfoxide; GP, generalized polarization; HA, hexanoic acid; HEPES, (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); HG, hesperetin glucoside; HGB, hesperetin glucoside 6″-*O*-butyrate; HGD, hesperetin glucoside 6″-*O*-decanoate; HGH, hesperetin glucoside 6″-*O*-hexanoate; HGL, hesperetin glucoside 6″-*O*-laurate; HGO, hesperetin glucoside 6″-*O*-octanoate; HGS, hesperetin glucoside 6″-*O*-stearate; LA, lauric acid; Laurdan, 6-dodecanoyl-2-dimethylamino-naphthalene; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide; NG, naringin; OA, octanoic acid; P, prunin; PB, prunin 6″-*O*-butyrate; PD, prunin 6″-*O*-decanoate; PH, prunin 6″-*O*-hexanoate; PI, propidium iodide; PL, prunin 6″-*O*-laurate; PO, prunin 6″-*O*-octanoate; PS, prunin 6″-*O*-stearate; SA, stearic acid; SDS, sodium dodecyl sulfate.

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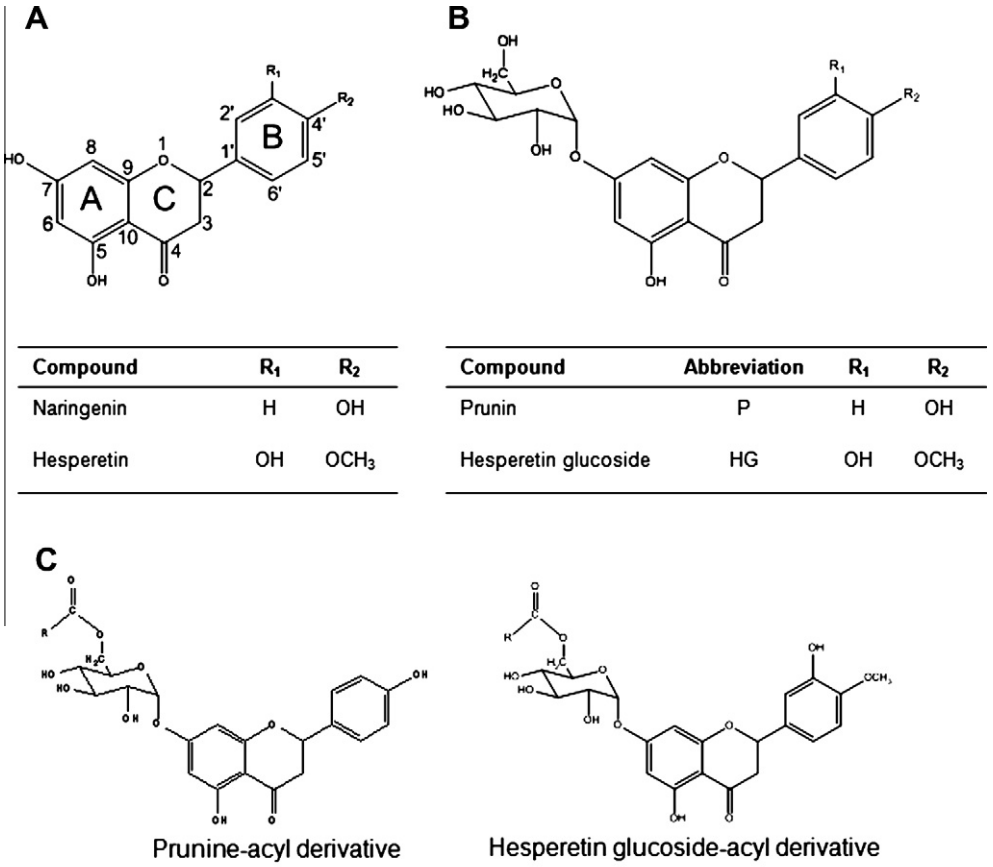

Compound	R_1	R_2
Naringenin	H	OH
Hesperetin	OH	OCH_3

Compound	Abbreviation	R_1	R_2
Prunin	P	H	OH
Hesperetin glucoside	HG	OH	OCH_3

R	Abbreviation	Prunin-alkyl ester	Hesperetin glucoside-alkyl ester
Butyric acid (C_4)	BA	PB	HGB
Hexanoic acid (C_6)	HA	PH	HGH
Octanoic acid (C_8)	OA	PO	HGO
Decanoic acid (C_{10})	DA	PD	HGD
Lauric acid (C_{12})	LA	PL	HGL
Stearic acid (C_{18})	SA	PS	HGS

Fig. 1. Structure of the compounds included in this study. (A) Naringenin (4′,5,7-trihydroxyflavanone); (B) Prunin and hesperetin glucoside; (C) Prunin- and hesperetin glucoside-alkyl esters.

these compounds are hydrophobic, and thus capable to spontaneously incorporate into lipid-reach environments, such as cell plasma membrane. Using a cell-free system, it has been demonstrated that P-alkyl esters retain the antioxidant capacity of the parent flavanone (Céliz and Daz, 2011).

Based on these precedents, we initiated the study of the biological effects of P- and HG-alkyl esters. In the current study we investigated the effects of a short term (1 h) exposure of human leukemia T cells (Jurkat cells) to micromolar concentrations of these compounds on cell viability, plasma membrane physical properties and their potential capacity to prevent the oxidative damage to cells challenged with two different oxidant molecules. Current results suggest that within the 0.1–10 μM range of concentrations, both P- and HG-alkyl esters can be considered safe to cultured human cells when used for short periods of time. However, at higher concentrations (100 μM) these compounds interfered with normal cell metabolism, decreasing mitochondrial activity. In addition, their antioxidant capacity in this experimental system depended on both the concentration of the compounds and the nature of the oxidant used to induce the insult.

2. Materials and methods

2.1. Chemicals

Alkyl acids (C_4, C_6, C_8, C_{10}, C_{12} and C_{18}), naringenin (4′,5,7-trihydroxyflavanone), bis-benzimide H (Hoechst 32258), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) and all other reagents were from the highest quality available and were purchased from Sigma-Aldrich (St. Louis, MO, USA). The fluorescent probes 5-(and-6)-carboxy-2′,7′-dichloro-dihydrofluorescein-diacetate (DCDCDHF), 4,4-difluoro-5-(4-phenyl-1,3-butadienyl)-4-bora-3a,4a-diaza-s-indacene-3-undecanoic acid (C_{11}-BODIPY), bis-(1,3-dibutylbarbituric acid) trimethine oxanol ($DiBaC_4(3)$), propidium iodide (PI), 6-(9-anthroyloxy)stearic acid (6-AS), 12-(9-anthroyloxy)stearic acid (12-AS), 16-(9-anthroyloxy) palmitic acid (16-AP) and 6-dodecanoyl-2-dimethylamino-naphthalene (Laurdan) were purchased from Invitrogen/Molecular Probes Inc. (Eugene, OR, USA). 2,2′-azobis-2-methyl-propani-