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Structure characteristics for intestinal uptake of flavonoids in Caco-2 cells

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ABSTRACT

Flavonoids are a large group of polyphenols and widely distributed in plant foods. Flavonoids exhibit various biological activities, such as anti-cancer, antioxidant and anti-inflammatory while poor oral bioavailability has been considered as a major hurdle in their use as functional foods. Cellular uptake and efflux of flavonoid implicates their bioavailability. To investigate the cellular uptake and efflux of flavonoids, 27 flavonoids were measured for their cellular uptake in Caco-2 cells with (CUV) and without (CU) the inhibitor of P-glycoprotein (P-gp) verapamil. Then, a quantitative structure-absorption relationship (QSAR) model containing 21 compounds as training set was obtained from their corresponding CU. The model showed good robustness and predictivity with a high cross-validation coefficient (Q^2) value of 0.809 and Log of the octanol/water partition coefficient (SlogP) and atomic charge on carbon 5 (Q_{C5}) were related to flavonoid uptake. The CUV of some flavonoids were significantly ($p < 0.05$ or $p < 0.01$) higher than their CU, suggesting that specific flavonoids are pumped out by P-gp. The structure-affinity relationship of flavonoids as substrates of P-gp was determined with the presence of 4′-OCH_3, 3′-OCH_3 and the absence of 3′-OH, 3-OH and 4′-OH favorable for the affinity of flavonoids. These results provide valuable information for screening flavonoids with good absorption and low affinity with transporters.

1. Introduction

Flavonoids are a large group of polyphenols that occur ubiquitously in plant foods. Flavonoids have attracted substantial attention due to their various biological activities including anti-cancer, antioxidant and anti-inflammatory (Cheng et al., 2011; Huang et al., 2011; Li, Liu, Zhang, & Yu, 2008). However, the flavonoid biopotency is not only determined by its activity, but also by absorption, distribution, metabolism, and excretion/toxicity (ADME/T) (Yang, Yang, Yuan, Wang, & Crans, 2004). Intestinal uptake and efflux are two major factors determining the bioavailability of orally administrated flavonoids. Poor oral bioavailability has been considered as a major hurdle in their use as drugs and nutraceuticals (Gonzales, Smagghe, et al., 2015). Therefore, it is essential to understand the molecular properties of flavonoids related to their oral bioavailability.

Generally, flavonoids are considered to penetrate the plasma membrane through passive diffusion (Glaeser, Bujok, Schmidt, Fromm, & Mandery, 2014; Sharma, Kanwal, Bhaskaran, & Gupta, 2014). The number and position of substituents such as hydroxylation (Tammela et al., 2004), methoxylation (Walle, 2009), prenyl groups (Chen, Zhao, Jia, & Hu, 2008; Murota et al., 2002) and glycosylation (Londoño-Londoño, Lima, Jaramillo, & Creczynski-pasa, 2010) on flavonoid basic structure also affects transport and increase or decrease in intestinal uptake. Properties such as lipophilicity or molecular weight (Chen et al., 2008) of flavonoids also play an important role in their absorption.

Caco-2 cells are widely used to evaluate intestinal absorption of drugs and nutraceuticals with various transporters such as P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), or multidrug resistance-associated protein 2 (MRP2) expressed in the apical and basolateral membranes. These transporters play an important role in preventing the uptake of xenobiotics (drugs and food components) from the gut into the body (Kimura et al., 2014; Tourniaire et al., 2005; Wang et al., 2009). Among the transporters, P-gp is one of the most important members of the ATP-binding cassette (ABC) transporter family both in human intestine and in Caco-2 cells (Wang, Cao, & Zeng,

Abbreviations: CU, cellular uptake of flavonoid without verapamil; CUV, cellular uptake of flavonoid with verapamil; SAR, structure activity relationship; QSAR, quantitative structure absorption relationship; MRP, multidrug resistance-associated protein; P-gp, P-glycoprotein; BCRP, breast cancer resistance protein; MEM, minimum essential medium; NEAA, non-essential amino acids; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; FBS, Fetal bovine serum; PLS, partial least squares; DFT, density functional methods; LSD, least significant difference

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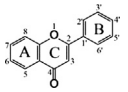
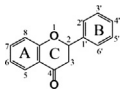
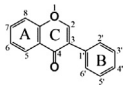
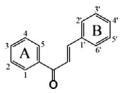
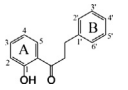
Table 1
The chemical structures of 27 flavonoids.

No	Flavonoids	Core structure	R_3	R_5	R_6	R_7	R_8	$R_{2'}$	$R_{3'}$	$R_{4'}$	$R_{5'}$
1	Flavone		H	H	H	H	H	H	H	H	H
2	Tangertin		H	OMe	OMe	OMe	OMe	H	H	OMe	H
3	Wogonin		H	OH	H	OH	OMe	H	H	H	H
4	Baicalein		H	OH	OH	OH	H	H	H	H	H
5[t]	Luteolin		H	OH	H	OH	H	H	OH	OH	H
6	Apigenin		H	OH	H	OH	H	H	H	OH	H
7	Chrysin		H	OH	H	OH	H	H	H	H	H
8	Galangin		OH	OH	H	OH	H	H	H	H	H
9	Quercetin		OH	OH	H	OH	H	H	OH	OH	H
10	Morin		OH	OH	H	OH	H	OH	H	OH	H
11	Kaempferol		OH	OH	H	OH	H	H	H	OH	H
12[t]	Kaempferide		OH	OH	H	OH	H	H	H	OMe	H
13	Isorhamnetin		OH	OH	H	OH	H	H	OMe	OH	H
14	Quercitrin		Orha	OH	H	OH	H	H	OH	OH	H
15	Hesperetin		H	OH	H	OH	H	H	OH	OMe	H
16	Naringenin		H	OH	H	OH	H	H	H	OH	H
17	Naringin		H	OH	H	ONG	H	H	H	OH	H
18	Liquiritigenin		H	H	H	OH	H	H	H	OH	H
19[t]	Taxifolin		OH	OH	H	OH	H	H	OH	OH	H
20	Formononetin		H	H	OH	H	H	H	OMe	H	H
21	Puerarin		–	H	H	OH	Cglc	H	H	OH	H
22	Glycitein		–	H	OMe	OH	H	H	H	OH	H
23	Daidzein		–	H	H	OH	H	H	H	OH	H
24[t]	Genistein		–	OH	H	OH	H	H	H	OH	H
25	Biochanin A		–	OH	H	OH	H	H	H	OMe	H
26[t]	Isoliquiritigenin		OH	OH	–	–	–	H	H	OH	H
27[t]	Neohesperidin dihydrochalcone		NG	OH	–	–	–	H	OH	OMe	H

Orha: −*O*-α-L-rhamnopyranosyl; ONG: −*O*-[6-Deoxy-α-L-mannopyranosyl]-β-D-glucopyranosyl); Cglc: −C-glucopyranosyl; [t]: Test set.

2005). The intestinal absorption of flavonoids has been investigated using Caco-2 cells (Wang et al., 2009). Flavonoids are known as substrates of P-gp which could account for the poor permeability of specific flavonoids (Lies, Martens, Schmidt, Boll, & Wenzel, 2012; Tourniaire et al., 2005). Verapamil, a P-gp inhibitor, has been used to examine the effects of P-gp on flavonoid uptake. Verapamil has been reported to inhibit other efflux transporters such as multidrug resistance-associated protein 1 (MRP1), which may also affect flavonoid permeability. However, MRP1 is rarely found in Caco-2 cells and its involvement in substrate efflux has been reported to be negligible (Aszalos, 2008).

The aim of this study was to investigate the uptake of flavonoids including determining the relationship between their physicochemical properties and uptake in Caco-2 cells. 27 flavonoids were tested including 7 flavones, 7 flavonols, 4 flavonones, 1 flavanol, 6 isoflavonoids and 2 chalcones (Table 1). The main objectives were (1) to evaluate the relationship between the structure and the cellular uptake of 27 flavonoids in Caco-2 cells by building a two dimensional quantitative structure-absorption relationship (2D-QSAR) model; (2) to study the structure affinity relationship of P-gp on the transport of flavonoids by adding the specific inhibitor verapamil. The structure characteristics which increase the uptake of flavonoids could improve the bioavailability of flavonoids. Moreover, flavonoids with structure characteristics which are unfavorable for affinity to P-gp will not be pumped out by P-gp, therefore their accumulation in cells will be increased and the bioavailability of flavonoids could also be improved.

2. Materials and methods

2.1. Chemicals

Human colon adenocarcinoma cell line Caco-2 (ATCC #HTB-37) was purchased from American Type Culture Collection (ATCC) (Rockville, MD, USA.). 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) was purchased from Gen-View Scientific Inc. (League, USA). Compounds **1** and **3** were purchased from Tianjinyifang Technology Co. Ltd. (Tianjin, China) (purity > 98%). The remaining flavonoids were purchased from Aladdin Chemistry Co., Ltd. (Shanghai, China) (purity > 98%). Verapamil hydrochloride, was purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan) (purity > 98%) and used as an inhibitor of P-gp. Colchicine was purchased from Sigma-Aldrich (St. Louis., MO, USA) and used as a substrate of P-gp. All the compounds were dissolved and diluted to a final concentration of 0.1% (v/v) DMSO. Fetal bovine serum (FBS) was obtained from Gibco Laboratories (Life Technologies Inc., Grand Island, NY, USA). Minimum essential medium (MEM) and non-essential amino acids (NEAA) were purchased from Hyclone (Logan, UT, USA). 12-well plates were purchased from Corning Costar (Cambridge, MA, USA). Bicinchoninic acid (BCA) protein assay kit was purchased from Dingguo changsheng Technology Co. Ltd. (Beijing, China).

2.2. Cell culture

Caco-2 cells were cultured in MEM supplemented with 10% FBS, 1% NEAA, penicillin (100 U/mL) and streptomycin (100 μg/mL) in an atmosphere of 5% CO_2 and 90% relative humidity at 37 °C. All cells used in this study were between passages 35 to 45.

2.3. MTT assay

The cytotoxicity of flavonoids and colchicine was evaluated by MTT assay. The cells were grown in 96-well plates at a density of 8×10^3 cells/well. After incubation with 40 μM flavonoid or colchicine for 24 h, the cells were further incubated with an MTT solution (0.5 mg/mL) for 4 h. Then, MTT solution was removed and DMSO