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Inhibitory effects and structure-activity relationship of flavonoids with respect to human organic anion-transporting polypeptides, OATP2B1

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Abstract

Organic anion-transporting polypeptides 2B1 (OATP2B1) have been implicated in the absorption of anionic drugs. Several plant flavonoids have been reported to inhibit the functions of OATP2B1, but the relationship between flavonoid structures and their OATP2B1-inhibitory effects is unknown. The present study aimed to investigate how flavonoids affect the functions of OATP2B1. Human embryonic kidney (HEK) 293 cells stably expressing human OATP2B1 were used to investigate the effects of 22 different flavonoids on the uptake of estrone-3-sulfate, which is a typical substrate of OATP2B1. The 50% inhibitory concentration (IC_{50}) was calculated and their relationship with lipophilicity (clogP), planar structure, and substituent groups was compared. The IC_{50} values for OATP2B1 of each flavonoid varied from 1.45 μM with cynaroside to 308 μM with flavone. The results showed that the OATP2B1-inhibitory effects were intensified by the addition of a hydroxyl group at the 4′, 3, and 3′ positions of flavonoids and that the OATP2B1-inhibitory effects were attenuated by methylation of a hydroxyl group at the 3′ and 4′ positions. Lipophilicity slightly affected some OATP2B1-inhibitory effects. Planar structure did not affect the OATP2B1-inhibitory effects. The effects of glycosylation on OATP2B1-inhibitory activity differed depending on the type of sugar added and the position to which it was added. Majority of the 22 different flavonoids investigated inhibited OATP2B1 functions, suggesting that the consumption of foods or health foods containing abundant flavonoids strongly inhibited OATP2B1 in the gastrointestinal tract and decreased the absorption of a substrate drug.

1. INTRODUCTION

Flavonoids are a class of polyphenolic compounds, and more than 6500 different flavonoids have been discovered. These flavonoids are abundant in vegetables, fruits, tea, wine, and other foods and drinks, and a recent study in the US estimated that the average daily intake of flavonoids is 344.83 mg.[1] Not only do flavonoids possess various physiological and pharmacological activities, such as anti-oxidant, anti-viral, and anti-immune activity,[2] but they have also been suggested to exhibit a preventive effect against cancer,[3] coronary heart disease,[4] bone loss,[5] and many other diseases associated with aging.[6] Because of the health-promoting actions and low toxicity of flavonoids, many plant-based supplements containing flavonoids have been marketed as health foods, and recently, increased interest in health has led to an annual increase in the number of people consuming these health products.[7, 8]

When plant-based supplements containing flavonoids are consumed, the flavonoids can have undesirable effects, particularly in the gastrointestinal tract, where they are present in high concentration. Small intestine epithelial cells express drug efflux transporters such as P-

glycoprotein (P-gp), multidrug resistance-associated protein 2 (MRP2), and breast cancer resistance protein (BCRP), as well as drug uptake transporters such as peptide transporter 1 (PEPT1).[9, 10, 11, 12] It was previously reported that the transport functions of P-gp,[13] MRP2,[14] BCRP,[15] and PEPT1[16] are inhibited by flavonoids, and that various flavonoid factors influence these inhibitory effects. For example, flavones inhibit the functions of P-gp and MRP2, but flavanones do not inhibit these effects. Flavones have a double bond between the 2 and 3 positions of the C ring, giving the molecule a planar structure, but flavanones do not have a double bond at this position; therefore, a planar structure cannot be formed. Thus, the planar structure in flavonoids is regarded to be important for P-gp and MRP2 inhibition.[17, 18] The P-gp-inhibitory effects of flavonoids have also been reported to show a significant positive correlation with the lipophilicity of the flavonoid.[17] The MRP2-inhibitory effects of flavonoids have not been found to have a significant positive correlation with flavonoid lipophilicity, but hydroxyl groups at the 3′ and 4′ positions have been implicated as important.[18] The glycosylation of flavonoids also reportedly attenuates their BCRP-inhibitory effects.[15, 19] As such, the inhibitory effects of flavonoids on transporter functions are expected to differ markedly depending on the flavonoid lipophilicity, planar structure, types and positions of substituents, glycosylation, and other factors.

Organic anion-transporting polypeptides (OATPs) transport a wide range of anionic compounds. OATP2B1 (also known as OTAP-B) is expressed on the luminal-side membrane of small intestine epithelial cells and have been implicated in the absorption of anionic drugs.[20] We previously reported that OATP2B1 functions are inhibited by naringin, naringenin, quercetin, and other such flavonoids found in grapefruit juice, by flavonols found in gingko leaf extract, and by catechins found in green tea extract.[21, 22] These previous studies showed that of the catechins, (-)-epicatechin gallate and (-)-epigallocatechin gallate, which have a gallate structure, show stronger inhibitory activity than (+)-catechin, (-)-epicatechin, and (-)-epigallocatechin, which do not have a gallate structure. Thus, structural differences between flavonoids may also have a major impact on OATP2B1-inhibitory activity. However, no previous report has investigated the relationships between flavonoid structures and OATP2B1 inhibitory effects.

The purpose of the present study was to investigate how OATP2B1 functions are affected by 22 different flavonoids with a flavone or flavanone skeleton, and determine which factors, including flavonoid lipophilicity, planar structure, and chemical structure, are important for the interactions between flavonoids and OATP2B1.

2. MATERIALS AND METHODS

2.1 Materials

Acacetin (5,7-dihydroxy-4′-methoxyflavone), chrysin (5,7-dihydroxyflavone), flavone, hesperetin (3′,5,7-trihydroxy-4′-methoxyflavanone), 7-hydroxyflavone, kaempferol (3,4′,5,7-tetrahydroxyflavone), naringenin (4′,5,7-trihydroxyflavanone), naringin (naringenin 7-neohesperidoside), quercetin (3,3′,4′,5,7-pentahydroxyflavone), quercitrin (quercetin 3-rhamnoside), and rutin (quercetin 3-rutinoside) were obtained from Sigma-Aldrich Corp. (St. Louis, MO, USA). Apigenin (4′,5,7-trihydroxyflavone), apigetrin (apigenin 7-glucoside), astragalin (kaempferol 3-glucoside), cynaroside (luteolin 7-glucoside), diosmetin (3′,5,7-trihydroxy-4′-methoxyflavone), eriodictyol (3′,4′,5,7,-tetrahydroxyflavanone), isorhamnetin (3,4′,5,7-tetrahydroxy-3′-methoxyflavone), isorhamnetin 3-glucoside, luteolin (3′,4′,5,7-tetrahydroxyflavone), miscanthoside (eriodictyol 7-glucoside), and nicotiflorin (kaempferol 3-rutinoside) were obtained from Extrasynthese S.A. (Genay, France). [^{3}H]Estrone-3-sulfate, ammonium salt (2.12 TBq/mmol) was procured from PerkinElmer, Inc. (Waltham, MA, USA). All other chemicals were commercial products of reagent grade.

2.2 Cell culture

Human embryonic kidney (HEK) 293 cells stably expressing human OATP2B1 (HEK/OATP-B cells) and HEK293 cells into which only a vector was introduced (HEK/Mock cells) were previously constructed in our lab.[21] These cells were cultured in Eagle's MEM medium containing 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, and 500 µg/mL G418 at 37°C and 5% CO_2/95% air.

2.3 Inhibition experiments

Uptake inhibition experimentation was performed as previously described[21, 22]. Briefly, the cells were seeded into 96-well plates at a density of 4–8 × 10^4 cells/well and cultured for two days. Next, the medium was replaced with medium that did not contain G418, and the uptake inhibition experiment was performed on the following day. After removing the medium, the cells were washed with 100 µL of uptake buffer (125 mM NaCl, 4.8 mM KCl, 5.6 mM D-glucose, 1.2 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM HEPES, pH 7.4), and then 200 µL of uptake buffer was added and they were pre-incubated for 10 minutes at 37°C. After the preincubation, the buffer was replaced with 100 µL of buffer containing [^{3}H] estrone-3-sulfate (10 nM) to initiate the uptake reaction. Each flavonoid was dissolved in dimethyl sulfoxide (DMSO) and diluted in uptake buffer at a concentration of 0.1–300 µL such that the final concentration of DMSO was 0.5% or lower. These uptake buffers were confirmed to have a pH of 7.4 and osmotic pressure of approximately 300 mOsm before they were used in the experiment. After 20 sec of incubation at 37°C, 200 µL of ice-cold uptake buffer (cold buffer) was added to stop uptake; the cells were then washed with 100 µL of cold buffer. Next, 200 µL of 1 N NaOH was added for solubilization, and then 100 µL of 2 N HCl for neutralization. A liquid scintillation cocktail was

added and the radioactivity was measured using a liquid scintillation counter. The protein mass of the cells was quantified by the Lowry method using bovine serum albumin as a standard.

The radioactivity taken up per protein mass of the cells (dpm/mg protein) was divided by the radioactivity concentration (dpm/μL) in the uptake buffer in order to calculate the uptake of [^{3}H] estrone-3-sulfate (μL/mg protein) into the cells; the value determined by subtracting the value obtained for the HEK/Mock cells from the value obtained for the HEK/OATP-B cells was considered to represent the OATP2B1-mediated uptake. OATP2B1-mediated uptake in the presence of flavonoids (U_I; % of control) was represented with the value in the control (0.5% DMSO) being 100%. The non-linear least square method (MLAB; Civilized Software, Bethesda, MD, USA) was used to apply the following formula to the results obtained in order to calculate the 50% inhibitory concentration (IC_{50} value; μM).

$$U_I = 100 - 100 \times I / (IC_{50} + I)$$

Where I is the concentration (μM) of the flavonoid.

2.4 Relationships between flavonoid structure and OATP2B1 inhibitory effect

A calculated value of the oil-water partition coefficient (clogP) from ACD/lab v11.02 was determined using SciFinder® (American Chemical Society, Washington DC, USA) as the lipophilicity index, and the relationship with the reverse logarithm of the IC_{50} (pIC_{50}) was investigated (SPSS 21; IBM Corp., Armonk, NY, USA). Next, IC_{50} values were compared between flavones and flavanones with the same substituents at the same positions (apigenin and naringenin, luteolin and eriodictyol, and diosmetin and hesperetin) to examine the role of the planar structure. Additionally, the relationships between IC_{50} values and the presence or absence of the substituents of each flavonoid (hydroxyl group, methoxy group, and glycoside) were examined.

Multiple regression analysis was performed (SPSS 21) using pIC_{50} values as dependent variables and with clogP values, planar structures, and substituents as independent values, with a dummy variable according to the stepwise method, in order to examine the effects of lipophilicity, planar structure, and substituents on inhibitory activity. The dummy variable was based on the structure of quercetin; the planar structure has a C2-C3 single bond (flavanone) and the substituents used were C3-H, C3-glucoside, C3-rhamnoside, C3-rutinoside, C5-H, C7-H, C7-glucoside, C7-neophesperidoside, C3′-H, C3′-methoxy, C4′-H, and C4′-methoxy.

3. RESULTS AND DISCUSSION

3.1 Inhibitory effects on OATP2B1 of each flavonoid

The 22 different flavonoids investigated in the present study exhibited a wide range of inhibitory effects on OATP2B1-mediated estrone-3-sulfate uptake (Fig. 1, Table 1). Cynaroside, apigetrin, isorhamnetin 3-glucoside, and astragalin exhibited very strong inhibitions ($IC_{50} \leq 10$ μM), followed by the strong inhibitory effects of luteolin, quercetin, naringin, isorhamnetin, eriodictyol, hesperetin, and kaempferol (10 μM < $IC_{50} \leq 30$ μM). Diosmetin, quercitrin, naringenin, apigenin, and miscanthoside exhibited moderate inhibitions (30 μM < $IC_{50} \leq 100$ μM), while 7-hydroxyflavone, rutin, nicotiflorin, acacetin, chrysin, and flavone had weak inhibitory effects (100 μM < IC_{50}).

The considerable differences in IC_{50} values between flavonoids suggest that additional factors are required for flavonoids to interact with OATP2B1. Therefore, we focused on flavonoid lipophilicity, planar structure, and chemical structure and investigated the factors critical for OATP2B1 inhibition by flavonoids.

3.2 Relationships between flavonoid structure and OATP2B1-inhibitory effect

3.2.1 Lipophilicity

Fig. 2 shows the relationship between the clogP value of each flavonoid and the pIC_{50} value for OATP2B1. The Pearson correlation coefficient between the clogP values and OATP2B1-inhibitory effects revealed a significant correlation for aglycone ($r = -0.601$, $p = 0.027$), but showed no correlation for glycoside ($r = 0.507$, $p = 0.163$) (Fig. 2). These results suggest that flavonoid lipophilicity is partially attributable to the differential OATP2B1 inhibition by flavonoids, but the following described substituents have a marked impact.

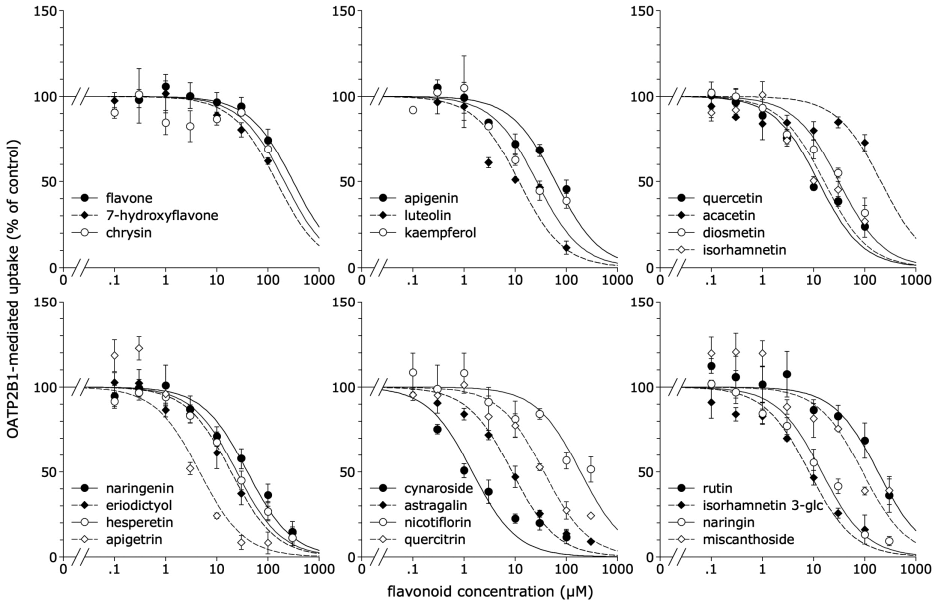

Figure - 1: Inhibitory effects of the 22 different flavonoids on the OTAP2B1 activity

The uptakes of 10 nM [^{3}H] estrone-3-sulfate into HEK/OATP-B cells and HEK/Mock cells were measured in the absence or presence of increasing concentration of flavonoids. The OATP2B1-mediated uptake was obtained by subtracting the uptake into HEK/Mock cells from that into HEK/OATP-B cells. Each point represents the mean ± S.E.M. of three experiments. The solid or dashed line represents the fitting line to calculate the 50% inhibitory concentration.

Table - 1: Chemical structures, lipophilicities, and OATP2B1 inhibitory effects of flavonoids used in this study

Flavonoids		Chemical Structure						clogP‡	IC_{50} (μM)§	$pIC_{50}$$
		2-3†	3	5	7	3′	4′			
a:	Flavone	=	H	H	H	H	H	3.818	308±48¶	3.51
b:	7-Hydroxyflavone	=	H	H	OH	H	H	3.455	146±15¶	3.83
c:	Chrysin	=	H	OH	OH	H	H	3.133	208±87¶	3.68
d:	Apigenin	=	H	OH	OH	H	OH	2.127	60.7±15.5	4.22
e:	Luteolin	=	H	OH	OH	OH	OH	2.695	11.8±3.7	4.93
f:	Kaempferol	=	OH	OH	OH	H	OH	2.685	26.4±7.2	4.58
g:	Quercetin	=	OH	OH	OH	OH	OH	1.989	12.3±2.5	4.91
h:	Acacetin	=	H	OH	OH	H	OCH_3	2.443	205±99¶	3.69
i:	Diosmetin	=	H	OH	OH	OH	OCH_3	2.877	31.0±6.2	4.51
j:	Isorhamnetin	=	OH	OH	OH	OCH_3	OH	2.787	16.0±4.5	4.80
k:	Naringenin	−	H	OH	OH	H	OH	2.628	39.8±6.3	4.40
l:	Eriodictyol	−	H	OH	OH	OH	OH	1.337	19.0±3.3	4.72
m:	Hesperetin	−	H	OH	OH	OH	OCH_3	1.938	24.5±3.2	4.61
n:	Apigetrin	=	H	OH	O-Glc	H	OH	−0.040	4.61±1.98	5.34
o:	Cynaroside	=	H	OH	O-Glc	OH	OH	0.527	1.45±0.42	5.84
p:	Astragalin	=	O-Glc	OH	OH	H	OH	1.212	8.67±1.10	5.06
q:	Nicotiflorin	=	O-Rut	OH	OH	H	OH	0.420	189±51¶	3.72
r:	Quercitrin	=	O-Rha	OH	OH	OH	OH	0.579	36.6±6.6	4.44
s:	Rutin	=	O-Rut	OH	OH	OH	OH	−0.903	182±37	3.74
t:	Isorhamnetin 3-glucoside	=	O-Glc	OH	OH	OCH_3	OH	0.805	8.15±1.65	5.09
u:	Naringin	−	H	OH	O-Neo	H	OH	−0.198	14.4±2.2	4.84
v:	Miscanthoside	−	H	OH	O-Glc	OH	OH	−0.745	90.9±38.3	4.04

†, single bond (−) or double bond (=) between 2 and 3 position; ‡, calculated using ACD/Labs Software; §, mean ± standard deviation; ¶, less than 50% inhibition of OATP2B1 activity even at maximum concentration used in this study; $, the negative logarithm of IC_{50} value; Glc, glucoside; Neo, neohesperidoside (rhamnoglucoside); Rha, rhamnoside; Rut, rutinoside (rhamnoglucoside).

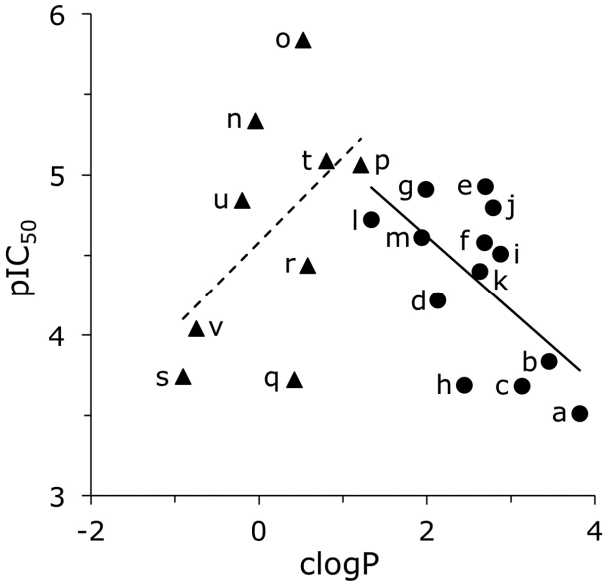

Figure - 2: Correlation between clogP of flavonoids and their IC_{50} for OATP2B1

Plot of the clogP value of each flavonoid and their pIC_{50} value for OATP2B1. The Pearson correlation coefficient for aglycone (solid circles) is −0.601 (p = 0.027, solid line) and for glycoside (solid triangles) is 0.507 (p = 0.163, dashed line). The alphabet a-v represent each flavonoid indicated in Table 2.

3.2.2 Planar structure

The IC_{50} values of apigenin, luteolin, and diosmetin, which have flavone skeletons, for OATP2B1 inhibition were similar to the IC_{50} values of naringenin, eriodictyol, and hesperetin, which have flavanone skeletons and have the same substituents at the same positions, respectively (the values were 60.7 μM vs. 39.8 μM, 11.8 μM vs. 19.0 μM, and 31.0 μM vs. 24.5 μM, respectively). Flavones have a double bond between the 2 and 3 positions of the C ring; therefore, the molecule forms a planar structure, but flavanones do not contain a double bond at this position, and thus, the molecule does not form a planar structure. Therefore, the dihedral angle between the C ring and B ring in a flavanone is much larger than the dihedral angle of a flavone.[18] It was previously reported that although flavones exhibit inhibitory action against P-gp, MRP2, and BCRP, flavanones do not exhibit inhibitory activity and do not have a planar structure, suggesting that the double bond between the 2 and 3 positions in the C ring is important for inhibition of these transporters by flavonoids.[17, 18, 23] In the present study, the inhibition strength of the flavones apigenin, luteolin, and diosmetin was similar to the inhibition strength of the flavanones naringenin, eriodictyol, and hesperetin, which have the same substituents at the same positions, respectively. This suggests that unlike P-gp, MRP2, and BCRP, the planar structure of flavonoid molecules is not important for OATP2B1 inhibition by flavonoids.

3.2.3 Substituents

The effect of changes in flavonoid substituents on their inhibitory effect on OATP2B1 was investigated using the IC_{50} values as an index (Table 2). Next, the inhibitory action against OATP2B1 (pIC_{50}) and the impact of lipophilicity, planar structure, and substituents were investigated by multiple regression analysis. The final model obtained by the backward elimination procedure is shown below.

$$pIC_{50} = 0.343\ (clogP) + 0.885\ (C3\text{-}Glc) + 1.19\ (C7\text{-}Glc) + 1.27\ (C7\text{-}Neo) - 0.411\ (C3'\text{-}H) - 1.16\ (C4'\text{-}H) - 0.473\ (C4'\text{-}OCH_3) + 4.05$$

$$p = 0.02;\ n = 22;\ R^2 = 0.638;\ s = 0.372;\ F = 6.30$$

Fig. 3 shows the relationships between the measured and predicted values.

Table - 2: Effects of hydroxylation, methylation and glycosylation of flavonoids on OATP2B1 inhibition activity

Change in chemical structure	Effect†	Change in OATP2B1 inhibition activity‡		
Hydroxylation				
7-position	↑	Flavone (308)	→	7-Hydroxyflavone (146)
5-position	↔	7-Hydroxyflavone (146)	→	Chrysin (208)
4′-position	↑	Chrysin (208)	→	Apigenin (60.7)
3-position	↑	Apigenin (60.7)	→	Kaempferol (26.4)
	↔	Luteolin (11.8)	→	Quercetin (12.3)
3′-position	↑	Apigenin (60.7)	→	Luteolin (11.8)
	↑	Naringenin (39.8)	→	Eriodictyol (19.0)
	↑	Apigetrin (4.61)	→	Cynaroside (1.45)
	↑	Kaempferol (26.4)	→	Quercetin (12.3)
	↔	Nicotiflorin (189)	→	Rutin (182)
	↑	Acacetin (205)	→	Diosmetin (31.0)
Methylation				
4′-position	↓	Apigenin (60.7)	→	Acacetin (205)
	↓	Luteolin (11.8)	→	Diosmetin (31.0)
	↔	Eriodictyol (19.0)	→	Hesperetin (24.5)
3′-position	↔	Quercetin (12.3)	→	Isorhamnetin (16.0)
Glycosylation (7-position)				
Glucoside	↑	Apigenin (60.7)	→	Apigetrin (4.61)
	↑	Luteolin (11.8)	→	Cynaroside (1.45)
	↓	Eriodictyol (19.0)	→	Miscanthoside (90.9)
Neohesperidoside	↑	Naringenin (39.8)	→	Naringin (14.4)
Glycosylation (3-position)				
Glucoside	↑	Kaempferol (26.4)	→	Astragalin (8.67)
	↔	Isorhamnetin (16.0)	→	Isorhamnetin 3-glucoside (8.15)
Rhamnoside	↓	Quercetin (12.3)	→	Quercitrin (36.6)
Rutinoside	↓	Kaempferol (26.4)	→	Nicotiflorin (189)
	↓	Quercetin (12.3)	→	Rutin (182)

†, Upwards arrows indicate that the IC_{50} value was decreased by less than half, downwards arrows indicate that the IC_{50} value was increased by more than twice, and left-right arrows indicate that the alternation of IC_{50} value was more than half and less than twice. ‡, The IC_{50} values are presented in parentheses.

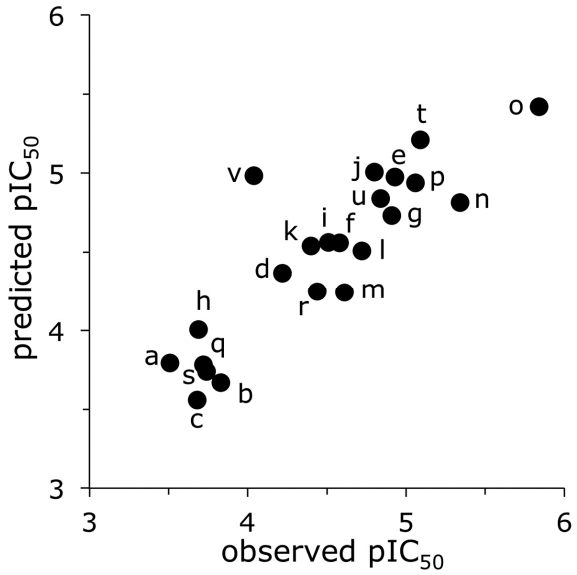

Figure - 3: Observed versus predicted IC_{50} plot for OATP2B1 inhibition by flavonoids

Predicted data were obtained by multiple regression analysis described in text. The alphabet a-v represent each flavonoid indicated in Table 2.

3.2.3.1 Hydroxyl group

With luteolin, eriodictyol, cynaroside, quercetin, and diosmetin, which contain a hydroxyl group bonded to the 3′ position of apigenin, naringenin, apigetrin, kaempferol, and acacetin, respectively, the IC_{50} value significantly decreased to half or less than half of the respective compound before the addition of a hydroxyl group (60.7 μM vs. 11.8 μM, 39.8 μM vs. 19.0 μM, 4.61 μM vs. 1.45 μM, 26.4 μM vs. 12.3 μM, and 205 μM vs. 31.0 μM, respectively). Similarly, the addition of a hydroxyl group at the 4′ or 3 position intensified inhibitory activity. In addition, multiple regression analysis showed that the coefficient for without a hydroxyl group at the 3′ position would have a negative value, and the IC_{50} would be high. These results suggest that hydroxyl groups at these positions are important for the intensification of OATP2B1-inhibitory activity.

3.2.3.2 O-methyl group

O-methylation of a hydroxyl group at the 4′ position suppressed the inhibitory activity. Acacetin and diosmetin, which are obtained by methylating the hydroxyl group at the 4′ position in apigenin and luteolin, showed weak inhibitory actions, with their IC_{50} values elevated significantly to double or more (60.7 μM vs. 205 μM, and 11.8 μM vs. 31.0 μM). In multiple regression analysis, the coefficient for a methoxy group at the 4′ position showed a negative value, which indicated that the IC_{50} would be high. These results are consistent with above mentioned results which hydroxyl groups at this positions are important for the intensification of OATP2B1-inhibitory activity.

3.2.3.3 O-glycoside

Apigetrin and cynaroside, which are obtained by binding a glucose to the hydroxyl group at the 7 position in apigenin, and luteolin, respectively, and naringin, which is obtained by binding a neohesperidose to the hydroxyl group at the 7 position of naringenin, showed intensified inhibitory actions with significantly decreased IC_{50} values (60.7 μM vs. 4.61 μM, 11.8 μM vs. 1.45 μM, and 39.8 μM vs. 14.4 μM, respectively) compared to aglycone. Astragalin, which are obtained by binding a glucose moiety to the hydroxyl group at the 3 position in kaempferol, showed intensified as compared to the respective aglycone (26.4 μM vs. 8.67 μM). In multiple regression analysis, a glucoside and a neophesperidoside at the 7 position had a positive coefficient, as did a glucoside at the 3 position. It is thus possible that adding a glucose moiety to a hydroxyl group at the 7 or 3 position strongly enhanced OATP2B1-inhibitory activity.

Quercitrin, which is obtained by binding a rhamnose to the hydroxyl group at the 3 position in quercetin, and nicotiflorin and rutin, which are obtained by binding a rutinose to the hydroxyl group at the 3 position in kaempferol and quercetin, respectively, showed attenuated inhibitory effects with significantly elevated IC_{50} values compared to the respective aglycone (12.3 μM vs. 36.6 μM, 26.4 μM vs. 189 μM, and 12.3 μM vs. 182 μM, respectively). These results demonstrate that the glycosylation of flavonoids does not necessarily enhance their OATP2B1-inhibitory activity; instead, it affects OATP2B1-inhibitory activity differentially depending on the type of sugar added and the position to which it is added. In case of OATP1B1, which belongs to the same OATP family, genistein and diosmetin significantly inhibited OATP1B1-mediated substrate uptake, whereas their glycosides genistin and diosmin did not inhibit substrate uptake. However, quercetin does not significantly affect OATP1B1-mediated substrate uptake, whereas its glycoside rutin does enhance substrate uptake.[24] Major changes in the steric structures of flavonoids caused by glycosylation may trigger complex interactions with OATP2B1 or OATP1B1.

4. CONCULUSION

In the present study, many of the 22 different flavonoids investigated inhibited the functions of OATP2B1. Adding a hydroxyl group to the 4′, 3, or 3′ position of a flavonoid enhanced its OATP2B1-inhibitory effect, and methylating the hydroxyl group at the 3′ or 4′ position was shown to attenuate its OATP-B-inhibitory effect. Flavonoids are abundant in various vegetables, fruits, and plant-derived health foods. With an increasing number of people consuming health foods in recent years, overconsumption of flavonoids may alter the dynamics of the endogenous and exogenous substrates of OATP2B1. Quercetin, which had an IC_{50} value of 11 μM in the present study, is reportedly consumed at a rate of 1 g daily as a supplement, with blood levels even reaching 10 μM[25, 26]; in such cases, the quercetin concentration in the gastrointestinal tract is expected to be even higher. Thus, it is possible that consumption of foods or health products rich in flavonoids strongly inhibit OATP2B1 in the gastrointestinal tract, lowering the blood levels of substrate drugs and leading to the failure of drug therapy.

5. ACKNOWLEDGEMENTS

All authors do not have any conflict of interest.

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