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Research Article

Inhibitory Effect of Flavonoids on the Efflux of *N*-Acetyl 5-Aminosalicylic Acid Intracellularly Formed in Caco-2 Cells

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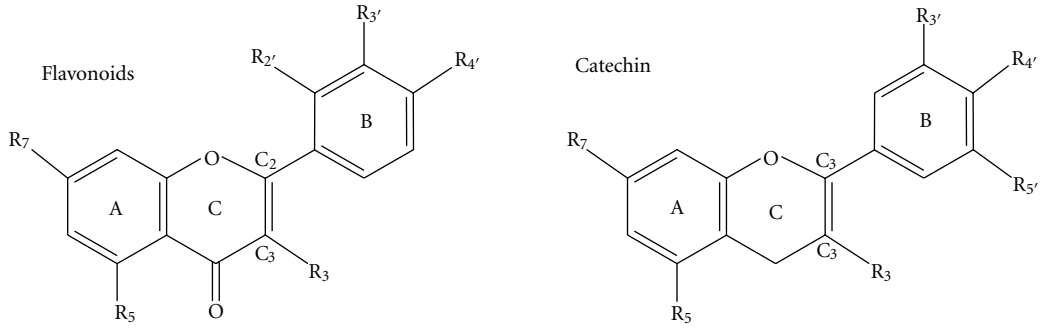
N-acetyl 5-aminosalicylic acid (5-AcASA) that was intracellularly formed from 5-aminosalicylic acid (5-ASA) at 200 μM was discharged 5.3, 7.1, and 8.1-fold higher into the apical site than into the basolateral site during 1, 2, and 4-hour incubations, respectively, in Caco-2 cells grown in Transwells. The addition of flavonols (100 μM) such as fisetin and quercetin with 5-ASA remarkably decreased the apically directed efflux of 5-AcASA. When 5-ASA (200 μM) was added to Caco-2 cells grown in tissue culture dishes, the formation of 5-AcASA decreased, and, in addition, the formed 5-AcASA was found to be accumulated within the cells in the presence of such flavonols. Thus, the decrease in 5-AcASA efflux by such flavonols was attributed not only to the inhibition of *N*-acetyl-conjugation of 5-ASA but to the predominant cellular accumulation of 5-AcASA. Various flavonoids also had both of the effects with potencies that depend on their specific structures. The essential structure of flavonoids was an absence of a hydroxyl substitution at the C5 position on the A-ring of flavone structure for the inhibitory effect on the *N*-acetyl-conjugation of 5-ASA, and a presence of hydroxyl substitutions at the C3′ or C4′ position on the B-ring of flavone structure for the promoting effect on the cellular accumulation of 5-AcASA. Both the decrease in 5-AcASA apical efflux and the increase in 5-AcASA cellular accumulation were also caused by MK571 and indomethacin, inhibitors of MRPs, but not by quinidine, cyclosporin A, P-glycoprotein inhibitors, and mitoxantrone, a BCRP substrate. These results suggest that certain flavonoids suppress the apical efflux of 5-AcASA possibly by inhibiting MRPs pumps located on apical membranes in Caco-2 cells.

1. Introduction

Sulfasalazine used in the therapy of inflammatory bowel diseases, such as ulcerative colitis and Crohn's disease [1, 2]. Ingested sulfasalazine passes to the colon without being absorbed in intestine and is split into 5-aminosalicylic acid (5-ASA) and sulfapyridine by colonic bacteria [1, 2]. Most of 5-ASA is metabolized by *N*-acetyl-conjugation in the form of *N*-acetyl 5-aminosalicylic acid (5-AcASA) in the colonic epithelia, while sulfapyridine is quickly absorbed from the colon and metabolized in the liver [3–5]. It has been proposed that 5-ASA, the active moiety of sulfasalazine, exerts an antiinflammatory activity by inhibiting prostaglandin synthesis in colonic mucosa [6, 7]. Some reports have shown that 5-AcASA has a potency as an inhibitor of prostaglandin synthesis comparable to that of 5-ASA [7], and therapeutically active when administered by enema to patients with ulcerative colitis [8]. However, 5-AcASA formed in colonic epithelia is immediately secreted into mucosal lumen and excreted in feces [9–11]. Thus, 5-AcASA is considered the portion that has already exerted therapeutical action within the bowel tissue [1–3, 9–11]. Zhou et al. reported that 5-AcASA was exclusively transported from the basolateral to the apical direction using human colon-derived Caco-2 cells [11]. However, the mechanism underlying the cellular transport of 5-AcASA has not extensively elucidated. It is well known that flavonoids (Figure 1), plant-derived compounds, alter the function of efflux transporters such as P-glycoprotein, that is, present in epithelium cells [12–14]. Recently, several researchers reported the inhibitory interaction of flavonoids with multidrug resistance-associated proteins (MRPs) that are responsible for active secretion of



Flavonoids	R_7	R_5	R_3	$R_{2'}$	$R_{3'}$	$R_{4'}$	$R_{5'}$	C_2–C_3
Galangin (Gal)	OH	OH	OH	—	—	—	—	Double
Kaempferol (Kam)	OH	OH	OH	—	—	OH	—	Double
Quercetin (Que)	OH	OH	OH	—	OH	OH	—	Double
Taxifolin (Tax)	OH	OH	OH	—	OH	OH	—	Single
Fisetin (Fis)	OH	—	OH	—	OH	OH	—	Double
Morin (Mor)	OH	OH	OH	OH	—	OH	—	Double
Geraldol (Ger)	OH	—	OH	—	OCH_3	OH	—	Double
Isorhamnetin (Iso)	OH	OH	OH	—	OCH_3	OH	—	Double
Chrysin (Chr)	OH	OH	—	—	—	—	—	Double
Apigenin (Api)	OH	OH	—	—	—	OH	—	Double
Luteolin (Lut)	OH	OH	—	—	OH	OH	—	Double
Diosmetin (Dio)	OH	OH	—	—	OH	OCH_3	—	Double
3-Hydroxyflavone (3-OH)	—	—	OH	—	—	—	—	Double
5-Hydroxyflavone (5-OH)	—	OH	—	—	—	—	—	Double
7-Hydroxyflavone (7-OH)	OH	—	—	—	—	—	—	Double
7,4′-Dihydroxyflavone (7,4′-OH)	OH	—	—	—	—	OH	—	Double
3′,4′-Dihydroxyflavone (3′,4′-OH)	—	—	—	—	OH	OH	—	Double
7,3′,4′-Trihydroxyflavone (7,3′,4′-OH)	OH	—	—	—	OH	OH	—	Double
Epicatechin (EC)	OH	OH	OH	—	OH	OH	—	Single
Epigallocatechin (EGC)	OH	OH	OH	—	OH	OH	OH	Single

Figure 1: Structure of flavonoids.

pharmacologically relevant drugs [15–20]. In this study, the effect of flavonoids and transporter inhibitors on the cellular efflux of 5-AcASA that was intracellularly formed from 5-ASA was examined in Caco-2 cells. Certain flavonoids and MRPs inhibitors displayed strong potency in decreasing the preferential apical efflux of 5-AcASA and in increasing the cellular accumulation of 5-AcASA in Caco-2 cells.

2. Materials and Methods

2.1. Materials. Materials and chemical reagents were purchased from the following companies: Transwells from Corning Costar (Cambridge, MA, USA); tissue culture dishes from Becton Dickinson Com. (Falcon; USA); flavonoids from Funakoshi Co. (Tokyo, Japan); 5-ASA and quinidine from Sigma-Aldrich Com. (Japan); MK571 from Alexis Biochemicals (Lausen, Switzerland); mitoxantrone from LKT Laboratories (MA, USA); indomethacin and other chemicals used from Wako Pure Chemical Co. (Osaka, Japan); and the Develosil RPAQUEOUS C-30-UG-3 column (4.6 I.D. × 150 mm) from Nomura Chemical Co. (Aichi, Japan). Cyclosporin A was purchased from Sigma-Aldrich Com. and Wako Pure Chemical Co. 5-AcASA was synthesized by the reaction of 5-ASA with acetic anhydride, as described by other researchers [21].

2.2. Efflux of 5-AcASA from Caco-2 Cells. Caco-2 cells were purchased from the Riken (no. RCB0988) and used as previously described [22]. The cell line was cultured in Dulbecco's modified Eagle's medium containing 12% fetal calf serum and penicillin-streptomycin-amphotericin B. The suspended cells were seeded on 6-well- polycarbonate Transwell inserts (0.4 μm mean pore size, 4.7 cm^2 growth area) at a density of 5×10^4 cells/dish, and then placed in an incubator in an atmosphere of 5% CO_2–95% air at 37°C. The Caco-2 cells in the Transwell were grown for 3 weeks in Dulbecco's modified Eagle's medium containing fetal calf serum. The monolayers with transepithelial electric resistance of more than 250 $\Omega\,cm^2$ were used for transport studies. 5-ASA in a stock solution at 50 mM was added to the apical chamber at a final concentration of 200 μM after 10 minutes of the addition of flavonols. After incubation for 2 and 4 hours at 37°C, 50 μL of the medium from both of the chambers was mixed with 50 μL of 0.5 M perchloric acid.

Table 1: The effect of flavonols and transporter inhibitors on the apical and basolateral efflux of *N*-acetyl 5-aminosalicylic acid in Caco-2 cells. Caco-2 cells grown in Transwells were incubated with 200 μM 5-ASA for 1, 2, and 4 hours in the presence of flavonols and transporter inhibitors at the concentration of 100 μM. Api: apical efflux of 5-AcASA, Baso: basolateral efflux of 5-AcASA. Each value represents the mean ± SD of four to five experiments.

		Control	Fisetin	Quercetin	Morin	MK571	Quinidine
1hr	Api (nmol)	1.01 ± 0.14	0.16 ± 0.06**	0.32 ± 0.07**	0.87 ± 0.03	0.47 ± 0.13**	0.91 ± 0.16
	Baso (nmol)	0.19 ± 0.02	0.31 ± 0.09**	0.47 ± 0.08**	0.43 ± 0.01**	0.45 ± 0.04**	0.18 ± 0.03
	Api/Baso	5.32 ± 0.38	0.52 ± 0.11**	0.68 ± 0.06**	2.02 ± 0.04**	1.04 ± 0.19**	5.06 ± 0.21
2hr	Api (nmol)	2.05 ± 0.28	0.36 ± 0.13**	0.80 ± 0.21**	1.64 ± 0.15	1.05 ± 0.05**	1.96 ± 0.59
	Baso (nmol)	0.29 ± 0.04	0.47 ± 0.11**	0.94 ± 0.07**	0.66 ± 0.06**	0.70 ± 0.03**	0.28 ± 0.07
	Api/Baso	7.07 ± 0.19	0.77 ± 0.16**	0.85 ± 0.16**	2.48 ± 0.04**	1.50 ± 0.11**	7.00 ± 0.18
4hr	Api (nmol)	5.04 ± 0.61	0.89 ± 0.25**	2.16 ± 0.35**	3.62 ± 0.94	2.30 ± 0.22**	4.61 ± 0.72
	Baso (nmol)	0.62 ± 0.08	0.97 ± 0.21**	2.05 ± 0.17**	1.15 ± 0.20*	1.14 ± 0.09**	0.59 ± 0.10
	Api/Baso	8.13 ± 0.27	0.92 ± 0.16**	1.05 ± 0.23**	3.15 ± 0.10**	2.02 ± 0.11**	7.81 ± 0.06

Significant difference from control $^*P < .05$, $^{**}P < .01$.

2.3. Cellular Accumulation of 5-AcASA. Caco-2 cell line at passage of 40 was used for the experiments. The suspended cells in Dulbecco's modified Eagle's medium containing 12% fetal calf serum and penicillin-streptomycin-amphotericin B were seeded on 35 mm plastic culture dishes at a density of 5×10^4 cells/dish. After seeding, the cells were cultured in a 37°C incubator under 5% CO_2–95% air at 37°C for two weeks until the cells were fully differentiated into confluent enterocyte-like monolayers. Flavonoids, 5-ASA and other chemicals were dissolved in dimethyl sulfoxide and added to the medium at definite concentrations, with the final concentration of dimethyl sulfoxide about 1%. After incubation for 2 hours, the cell monolayers were washed twice with Hanks balanced solution and harvested. The adequate volume of the medium and cell suspensions was treated with the same volume of 0.5 M perchloric acid.

2.4. HPLC Analysis. Chromatographic separation and quantitative determination were carried out according to the HPLC analytical methods described previously [23]. A 0.1 mL aliquot of perchloric acid-treated sample was neutralized with 25 μL of 1 M NaOH solution and 25 μL of 0.5 M Tris-HCl buffer (pH 7.4), and the total volume was adjusted to 0.5 mL with HPLC elution solvent. A 50 μL aliquot of sample was injected onto a Develosil C-30-UG-3 (4.6 I.D. × 150 mm) column adjusted to 40°C, and 5-AcASA was separated by solution with a mixture of acetonitrile (4%) and 20 mM phosphate buffer (pH 5.0 solution) using a CCPD HPLC system equipped with an FS-8020 fluorescence detector (Tosoh Co., Japan). The flow rate of the mobile phase was 1.0 mL/min, and elution of 5-ASA and 5-AcASA was monitored at a fluorescence excitation wavelength of 310 nm and an emission wavelength of 480 nm. 5-ASA and 5-AcASA were eluted at 2.7 and 11.5 minutes, respectively. The quantitative determination of 5-AcASA was based upon the integration of fluorescence peak areas.

2.5. Statistical Analysis. The data in figures are given as the mean ± S.D. of four to five experiments. Differences among the mean values were assessed by Dunnett's test using Stat-100 (BIOSOFT, UK) or Student's *t*-test. A *P* value of < 0.05 was considered significant.

3. Results

The incubation of Caco-2 cells with 5-ASA formed only one peak of 5-ASA metabolite, which was identified as 5-AcASA by the same retention time as the synthesized standard in HPLC. The *N*-acetyl-conjugative reaction of 5-ASA in Caco-2 cells was saturated above 1 mM of 5-ASA. The effect of flavonols and inhibitors of transporters on 5-AcASA efflux was examined using Caco-2 cell monolayers grown in Transwells which contained 1.5 and 2.6 mL Dulbecco's modified Eagle's medium in the apical and basolateral chambers, respectively. 5-ASA was loaded at 200 μM in the apical chamber and 5-AcASA discharged from both of the apical and basolateral sites was measured. After 1, 2, and 4-hour incubation, amounts of 5-AcASA were 1.01, 2.05, and 5.04 nmoles in the apical chamber and 0.19, 0.29, and 0.62 nmoles in the basolateral chamber, respectively (Table 1). The apical efflux of 5-AcASA was 5.32, 7.07, and 8.13-fold higher than the basolateral efflux at 1, 2, and 4-hour incubation, respectively. When fisetin and quercetin (100 μM) were added with 5-ASA to Caco-2 cells, the apical efflux of 5-AcASA decreased remarkably (Table 1). The basolateral efflux of 5-AcASA rather increased in the presence of such flavonols. The ratios for the apical to the basolateral efflux of 5-AcASA actually decreased to 0.52 and 0.68 at 1 hour, 0.77 and 0.85 at 2 hours, and 0.92 and 1.05 at 4-hour incubation, in the presence of fisetin and quercetin, respectively. Morin had a weaker effect than fisetin and quercetin. MK571, a MRPs inhibitor, showed a similar effect to quercetin; however, quinidine, a P-glycoprotein inhibitor, had no effects.

Figure 2 shows the time course of the amount of 5-AcASA in the cells, medium, and their total (cells plus medium), and the percentage of cellular accumulation of 5-AcASA at 1, 2, and 4-hour incubation in the presence of flavonols (100 μM) with 5-ASA (200 μM) in Caco-2 cells

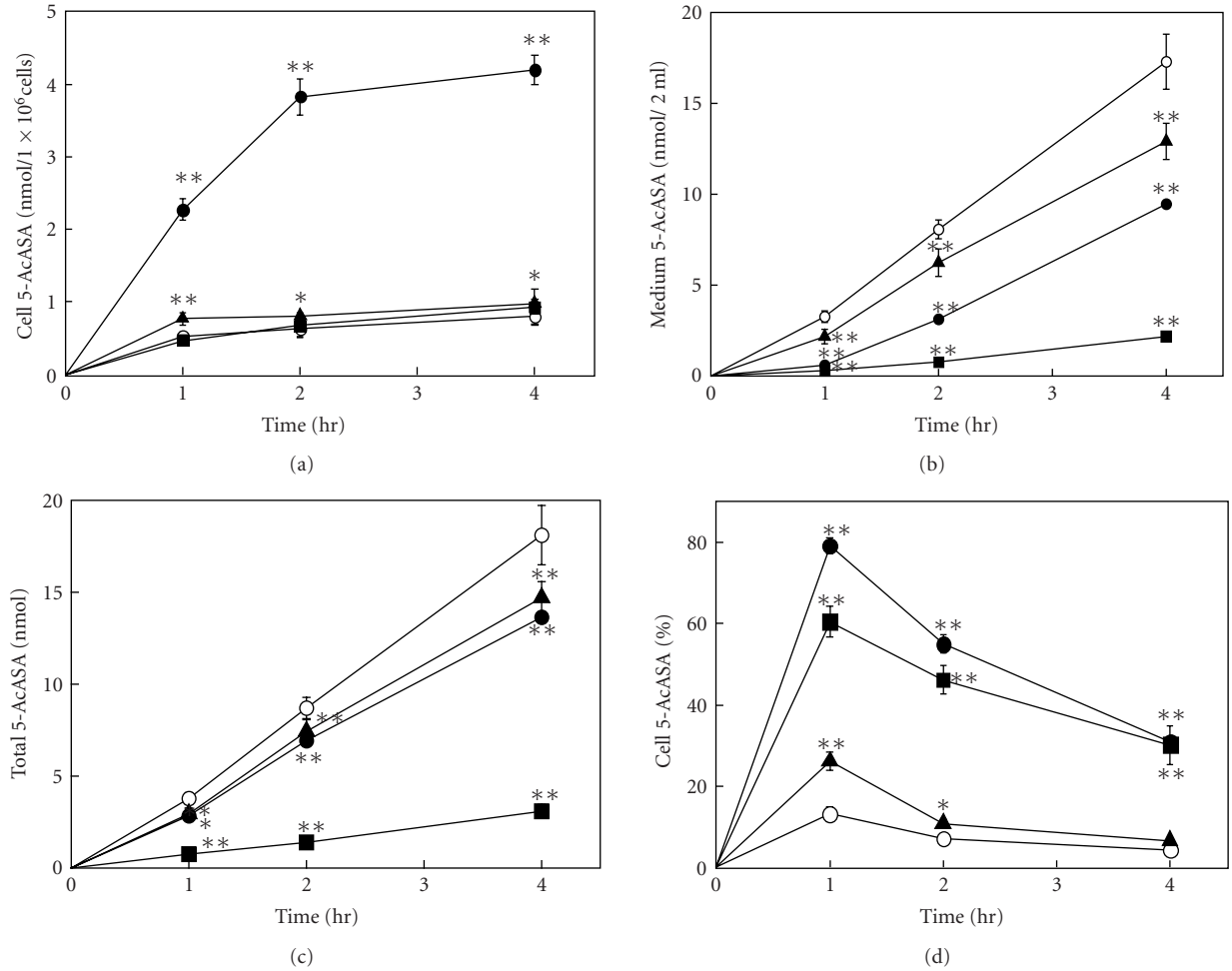


FIGURE 2: (a) The time course curve of *N*-acetyl 5-aminosalicylic acid in Caco-2 cells, (b) the medium and (c) the total, and (d) the cellular accumulation percent. Caco-2 cells grown in tissue culture dishes were incubated with 200 μM 5-ASA for 1, 2, and 4 hours in the absence (○) and the presence of quercetin (●), fisetin (■), and morin (▲) at the concentration of 100 μM. Cell 5-AcASA (%): (cells/cells plus medium) × 100. Each point represents the mean ± SD of four to five experiments. Significant difference from control $^{*}P < .05$, $^{**}P < .01$.

grown in tissue culture dishes. 5-AcASA was formed at the rate of 4 nmol/h/1 × 10^6 cells during a 4 h-incubation period in the control cells. Flavonoids are potent inhibitors of *N*-acetyltransferase [23]. Fisetin remarkably decreased the formation of 5-AcASA from 5-ASA in Caco-2 cells. Furthermore, a large amount of 5-AcASA was found in the cells treated by quercetin. The amount of 5-AcASA inside the control cells was 12 percent of the total 5-AcASA at a 1 h-incubation and decreased to 6.3 and 3.2 percents at 2 and 4-hour incubation, respectively. The cellular accumulation rate increased by several-fold than that of the control cells by quercetin and fisetin, and increased slightly by morin during a 4 h-incubation period. Figure 3 shows the amount of 5-AcASA in the cells and medium in the presence of various flavonoids at a 2 h-incubation. Flavonoids that lack a hydroxyl substitution at the C5 position on the A-ring had a strong inhibitory effect on the *N*-acetyl-conjugation of 5-ASA. The total 5-AcASA formed in the presence of fisetin, 7,3′,4′-OH flavone, 7,4′-OH flavone and geraldol decreased to 16.3, 23.3, 54.3, and 68.3 percents of that of the control cells, respectively. Furthermore, most of flavonols and flavones caused an abundant cellular accumulation of 5-AcASA inside the cells. The cellular 5-AcASA accumulation was 52.7 percent of the total formed in the presence of quercetin, the most effective one among flavonoids tested (Table 2). Flavonoids that lack a C2-3 double bond or a carboxyl group at the C4 position on the C-ring, such as catechins and taxifolin, had no effects. The structural feature required for the potent effect on the cellular 5-AcASA accumulation was a presence of hydroxyl group on the B-ring of flavone structure. The effect of inhibitors or substrate of transporters on the cellular 5-AcASA accumulation was compared with flavonols at a 2 h-incubation with 200 μM of 5-ASA in Caco-2 cells (Figure 4). MK-571 and indomethacin, MRPs inhibitors [24–26], increased in concentration-dependent manner the cellular 5-AcASA accumulation, while they did not affect the formation of 5-AcASA. MK-571 was more effective than indomethacin and showed equivalent efficacy to quercetin and fisetin. On the other hand, qunidine, a

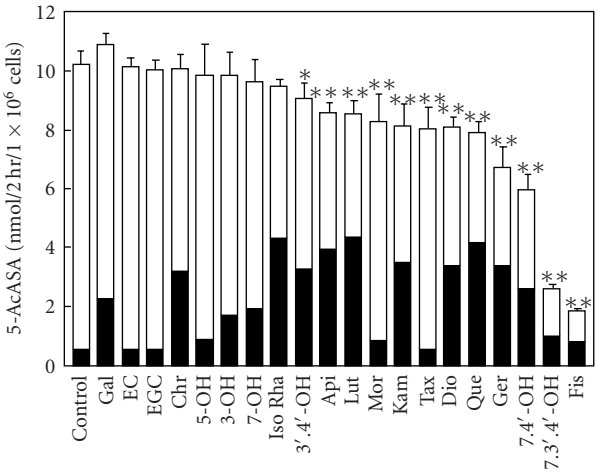


FIGURE 3: The amount of *N*-acetyl 5-aminosalicylic acid in Caco-2 cells and the medium. Caco-2 cells grown in tissue culture dishes were incubated with 200 μM 5-ASA for 2 hours in the presence of flavonoids at the concentration of 100 μM. 5-ASA in cells (Closed column), 5-ASA in medium (Open column). Each bar represents the mean ± SD of four to five experiments. Significant difference from control $^{*}P < .05$, $^{**}P < .01$.

P-glycoprotein inhibitor, and cyclosporine A, an inhibitor of both P-glycoprotein and MRPs [27, 28], did not affect the cellular 5-AcASA accumulation. Mitoxantrone, a breast cancer resistance protein (BCRP) substrate [29], had no effects either at the concentration of 20 μM (data not shown).

4. Discussion

5-AcASA that was formed from 5-ASA in the interior of cells was discharged preferentially to the apical direction compared to the basolateral direction in Caco-2 cells grown in Transwells. Quercetin and fisetin remarkably decreased the apical efflux of 5-AcASA, while morin did with a less potency. The amount of 5-AcASA in Caco-2 cells and the medium was measured during a 4 h-incubation with 5-ASA in the presence of such flavonols. Flavonoids are effective inhibitors of *N*-acetyl-conjugation of 5-ASA in rat liver cytosol preparation [23]. Fisetin, in particular, exhibited strong inhibitory activity on 5-AcASA formation in Caco-2 cells. Thus, the inhibition of 5-AcASA formation is likely to contribute largely to the decrease in the 5-AcASA efflux in the case of fisetin. However, quercetin showed a much weaker inhibitory effect on the 5-AcASA formation than fisetin. Surprisingly, the formed 5-AcASA was found to be accumulated inside the cells treated by flavonols. For quercetin, the cellular accumulation of 5-AcASA coincides with the decrease in 5-AcASA apical efflux. An increase in the basolateral efflux of 5-AcASA during an incubation of Transwells is probably due to the extensive cellular accumulation of 5-AcASA particularly in quercetin-treated cells.

A large group of flavonoids were examined for their inhibitory effects on the 5-AcASA formation as well as their promoting effects on the cellular 5-AcASA accumulation. A key chemical determinant necessary for exerting the strong inhibitory effect on the *N*-acetyl-conjugation of 5-ASA was a lack of hydroxyl substitution at the C5 position on the A-ring of flavone structure such as fisetin and 7,3′,4′-OH favone. On the other hand, the structural requirement for the promoting effect on cellular 5-AcASA accumulation was a presence of hydroxyl substitution at the C3′ or C4′ position on the B-ring of flavone structure. Therefore, the inhibition of 5-AcASA formation and the promotion of cellular 5-AcASA accumulation by flavonoids seem to be caused by different mechanisms.

The results mentioned above suggest that 5-AcASA is pumped out by an active efflux transporter located on the apical membrane and certain flavonoids appear to play an important replacing role in the apical-directed transport of 5-AcASA in Caco-2 cells. Flavonoids are well-known modulators of the cellular transport of various substances mediated by P-glycoprotein which is localized on apical membranes in polarized cells [12–14]. Recently, several researchers reported the interaction of flavonoids with MRPs transporters. Walgren et al. reported that the efflux of quercetin 4′-beta-glucoside across Caco-2 cell monolayers was mediated by MRP2 [24]. Van Zanden et al. studied on the inhibitory effect of quercetin on MRPs pump-mediated efflux of calcein and vincristine, well-known MRPs substrates, in the MRP1 and MRP2 transfected MDCK cells [18–20]. They mentioned that MRP2 displayed higher selectivity for flavonoid-type inhibition than MRP1. Phase II metabolites of various drugs conjugated to glutathione, glucuronate, or sulfate are generally considered to be transported by MRPs-like transporters [30–32]. MRPs were characterized as the canalicular multispecific organic anion transporters that function in terminal secretion into bile canaliculus of endo- and xenobiotics such as acetaminophen metabolites, bilirubin glucuronides, 2,4-dinitrophoenyl-S-glutathione, 17β-glucuronosyl estradiol, and 4-methylumbelliferyl glucuronide that are conjugated in hepatocytes [33–35]. The transcellular transport of acetyl-conjugated 5-ASA from the basolateral site to the apical site in Caco-2 cell was first reported by Zhou et al. [11]. However, the transporter-mediated efflux of 5-AcASA has not been investigated thoroughly. To address the interest in involvement of transporters that are responsible for the 5-AcASA apical efflux in Caco-2 cells, several inhibitors of transporters were examined for their suppressing effect on the 5-AcASA apical efflux and promoting effect on the cellular 5-AcASA accumulation. MK571 and indomethacin, inhibitors of MRPs had similar effects to flavonoids. Quinidine, a P-glycoprotein inhibitor, and Cyclosporine A, an inhibitor of P-glycoprotein and MRPs [27, 28], showed no effects. Absence of inhibitory activity of Cyclosporine A may be explained by substrate specificity of 5-AcASA for MRPs. Mitoxantrone, a substrate of BCRP [29], had no effects either. These results suggest that 5-AcASA is possibly pumped out by an MRPs-like transporter and certain flavonoids inhibit their efflux-pump activity in Caco-2 cells.

Flavonoids are part of the human diet and possess many health benefits with low toxicity [36, 37]. However, flavonoids are poorly absorbable compounds from the

Table 2: The cellular accumulation percent of in *N*-acetyl 5-aminosalicylic acid Caco-2 cells. Caco-2 cells grown in tissue culture dishes were incubated with 200 μM 5-ASA for 2 hours in the presence of flavonoids at the concentration of 100 μM. Cellular accumulation percent: (cells/cells plus medium) × 100. Each value represents the mean ± SD of four to five experiments.

Flavonoids	Cellular accumulation (%)	Flavonoids	Cellular accumulation (%)
Control	5.5 ± 0.8	7,3′,4′- OH flavone	38.7 ± 4.2**
Epicatechin	5.7 ± 0.3	Diosmetin	41.8 ± 2.4**
Epigallocatechin	5.8 ± 0.3	Fisetin	42.7 ± 1.2**
Taxifolin	6.9 ± 0.6	7,4′-OH flavone	42.8 ± 4.5**
5-OH flavone	9.2 ± 0.2**	Kaempferol	43.1 ± 0.7**
Morin	9.9 ± 0.3**	Isorhamnetin	45.4 ± 4.1**
3-OH flavone	17.4 ± 0.9**	Apigenin	45.7 ± 2.3**
7-OH flavone	20.1 ± 3.8**	Geraldol	50.2 ± 1.1**
Galangin	21.3 ± 3.1**	Luteolin	50.7 ± 3.2**
Chrysin	31.7 ± 3.5**	Quercetin	52.7 ± 2.5**
3′,4′-OH flavones	36.7 ± 3.6**		

Significant differences from control $^{*}P < .05$, $^{**}P < .01$.

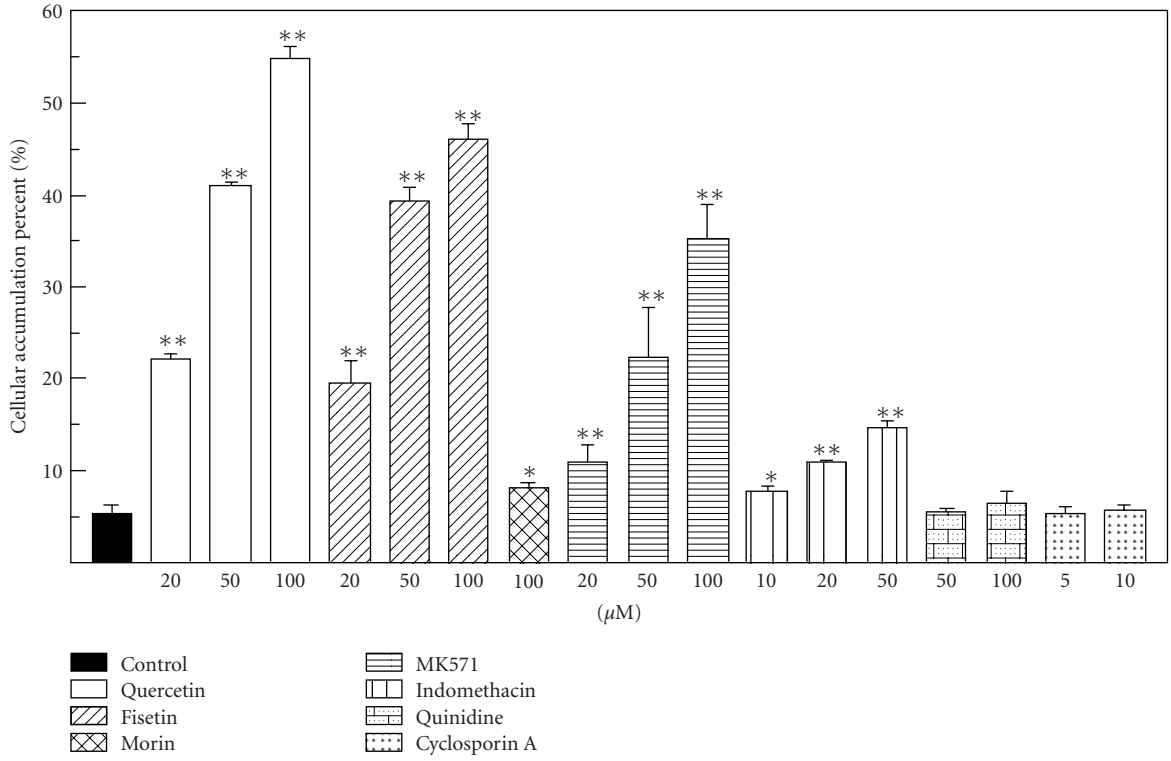


Figure 4: The effect of flavonols and transpoter inhibitors on the cellular accumulation of *N*-acetyl 5-aminosalicylic acid in Caco-2 cells. Caco-2 cells grown in tissue culture dishes were incubated with 200 μM 5-ASA for 2 hours in the presence of flavonols and transporter inhibitors at the concentration of 100 μM. Cellular accumulation percent : (cells/cells plus medium) × 100. Each bar represents the mean ± SD of four to five experiments. Significant difference from control $^{*}P < .05$, $^{**}P < .01$.

digestive tract in vertebrates [38, 39]. When quercetin was given p.o. to the rats (630 mg/kg), approximately 20% of the total dose was absorbed from the digestive tract, more than 30% was decomposed in the intestinal microflora, and approximately 30% was excreted unchanged in the feces during 72 hours [38]. After a single oral dose of quercetin in humans (4 g), approximately 53% of the dose was recovered unchanged in the feces. Thus it was concluded that 1% of the original 4 g dose of quercetin was absorbed [39]. In this study, flavonoids were added at the concentration range from 20 to 100 μM only into the apical compartment of Caco-2 cells in Transwells that faces to intestinal lumen in vivo. A high luminal level around 100 μM of flavonoids is expected to be achieved with a single oral administration of a few hundred mg of flavonoids in humans.

5-ASA, an active moiety of sulfasalazine, is immediately secreted into the luminal side from intestinal epithelia following extensive *N*-acetyl-conjugation, and is finally excreted into feces [3–5]. Zhou et al. [11] reported that at luminal levels below 200 μg/mL (concentrations that are typically achieved by controlled release dosage forms), intestinal secretion of 5-AcASA accounts for more than 50% of the total 5-ASA elimination. Thus, 5-AcASA has been considered to be therapeutically nonactive portion [1–3, 9–11]. However, 5-AcASA has still antiinflammatory potential if the drug retains within the intestinal tissues [8]. The efficacy of 5-ASA therapy correlates with tissue delivery of 5-ASA, that is, determined by *N*-acetylation and cellular discharge. The present study showed that certain flavonoids have the inhibitory effect on *N*-acetyl-conjugation of 5-ASA and the suppressive effect on the 5-AcASA apical efflux in Caco-2 cells. Viewed in this light, both of these effects of flavonoids seem to be desirable in the treatment of inflammatory bowel diseases, since coadministration of flavonoids with 5-ASA is expected to increase the tissue levels of 5-ASA and 5-AcASA in intestine.

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