

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.

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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 therapeu-

tically 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 therapeutic 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 cm² growth area) at a density of 5 × 10⁴ cells/dish, and then placed in an incubator in an atmosphere of 5% CO₂–95% 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 Ω cm² 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 μ M 5-ASA for 1, 2, and 4 hours in the presence of flavonols and transporter inhibitors at the concentration of 100 μ M. Api: apical efflux of 5-AcASA, Baso: basolateral efflux of 5-AcASA. Each value represents the mean \pm SD of four to five experiments.

| | | Control | Fisetin | Quercetin | Morin | MK571 | Quinidine |
|-----|-------------|-----------------|-------------------|-------------------|-------------------|-------------------|-----------------|
| 1hr | Api (nmol) | 1.01 \pm 0.14 | 0.16 \pm 0.06** | 0.32 \pm 0.07** | 0.87 \pm 0.03 | 0.47 \pm 0.13** | 0.91 \pm 0.16 |
| | Baso (nmol) | 0.19 \pm 0.02 | 0.31 \pm 0.09** | 0.47 \pm 0.08** | 0.43 \pm 0.01** | 0.45 \pm 0.04** | 0.18 \pm 0.03 |
| | Api/Baso | 5.32 \pm 0.38 | 0.52 \pm 0.11** | 0.68 \pm 0.06** | 2.02 \pm 0.04** | 1.04 \pm 0.19** | 5.06 \pm 0.21 |
| 2hr | Api (nmol) | 2.05 \pm 0.28 | 0.36 \pm 0.13** | 0.80 \pm 0.21** | 1.64 \pm 0.15 | 1.05 \pm 0.05** | 1.96 \pm 0.59 |
| | Baso (nmol) | 0.29 \pm 0.04 | 0.47 \pm 0.11** | 0.94 \pm 0.07** | 0.66 \pm 0.06** | 0.70 \pm 0.03** | 0.28 \pm 0.07 |
| | Api/Baso | 7.07 \pm 0.19 | 0.77 \pm 0.16** | 0.85 \pm 0.16** | 2.48 \pm 0.04** | 1.50 \pm 0.11** | 7.00 \pm 0.18 |
| 4hr | Api (nmol) | 5.04 \pm 0.61 | 0.89 \pm 0.25** | 2.16 \pm 0.35** | 3.62 \pm 0.94 | 2.30 \pm 0.22** | 4.61 \pm 0.72 |
| | Baso (nmol) | 0.62 \pm 0.08 | 0.97 \pm 0.21** | 2.05 \pm 0.17** | 1.15 \pm 0.20* | 1.14 \pm 0.09** | 0.59 \pm 0.10 |
| | Api/Baso | 8.13 \pm 0.27 | 0.92 \pm 0.16** | 1.05 \pm 0.23** | 3.15 \pm 0.10** | 2.02 \pm 0.11** | 7.81 \pm 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% CO₂-95% 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 μ L of 1 M NaOH solution and 25 μ L 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 μ L aliquot of sample was injected onto a Develosil C-30-UG-3 (4.6 I.D. \times 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 \pm 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 μ M 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 μ M) 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 μ M) with 5-ASA (200 μ M) in Caco-2 cells

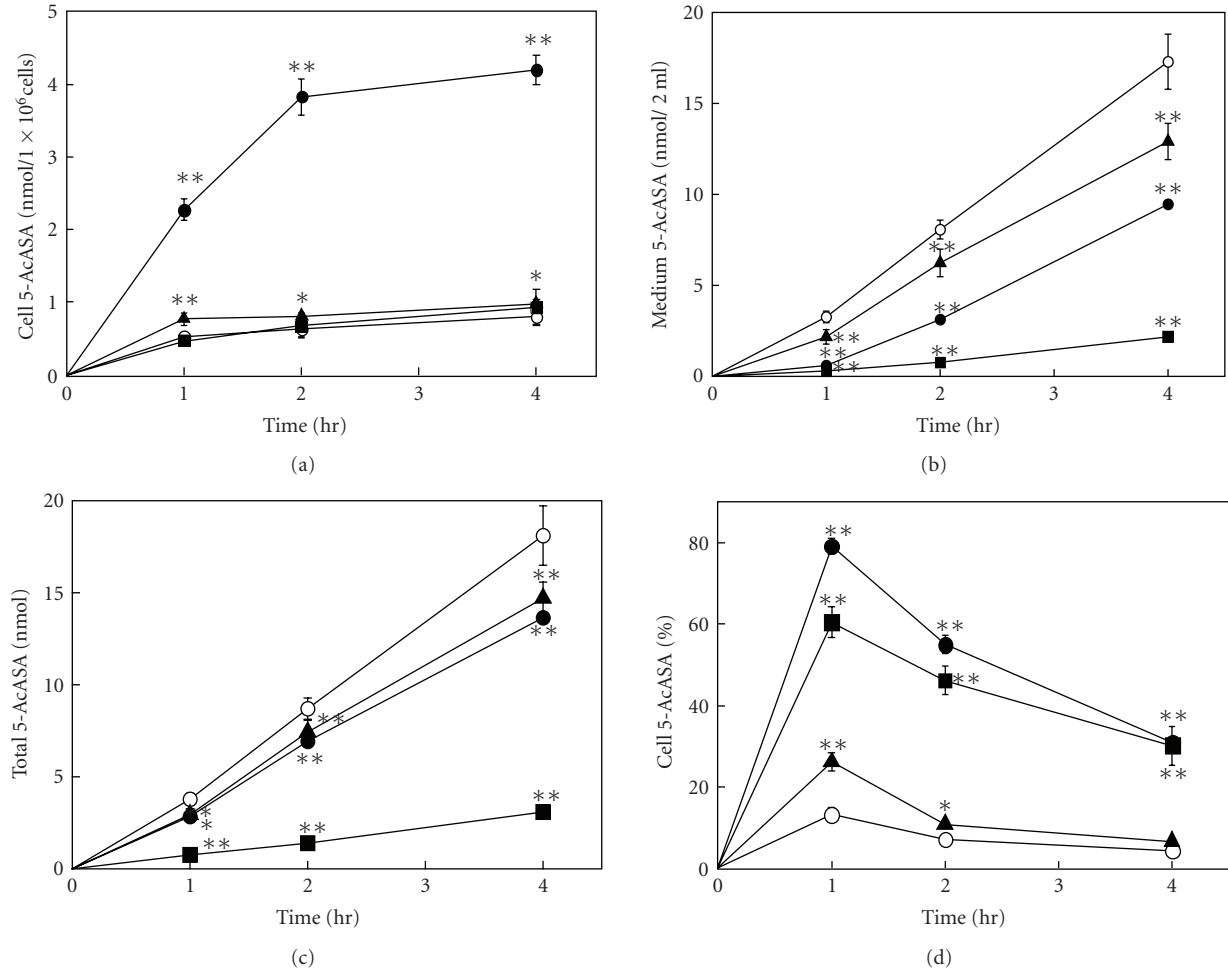


FIGURE 2: (a) The time course curve of *N*-acetyl 5-aminosalicylic acid in Caco-2 cells, (b) the medium and (c) the total, and (d) the cellular accumulation percent. Caco-2 cells grown in tissue culture dishes were incubated with 200 μ M 5-ASA for 1, 2, and 4 hours in the absence (○) and the presence of quercetin (●), fisetin (■), and morin (▲) at the concentration of 100 μ M. Cell 5-AcASA (%): (cells/cells plus medium) \times 100. Each point represents the mean \pm 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 \times 10⁶ 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 μ M 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, quinidine, a

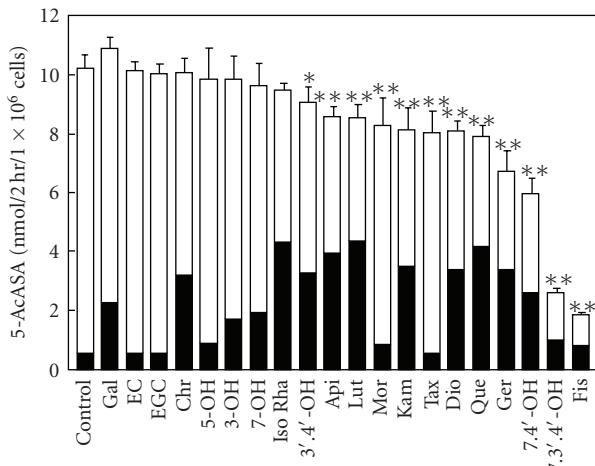


FIGURE 3: The amount of *N*-acetyl 5-aminosalicylic acid in Caco-2 cells and the medium. Caco-2 cells grown in tissue culture dishes were incubated with 200 μ M 5-ASA for 2 hours in the presence of flavonoids at the concentration of 100 μ M. 5-ASA in cells (Closed column), 5-ASA in medium (Open column). Each bar represents the mean \pm 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 μ M (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 calcine 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 multi-specific organic anion transporters that function in terminal secretion into bile canaliculus of endo- and xenobiotics such as acetaminophen metabolites, bilirubin glucuronides, 2,4-dinitrophenyl-S-glutathione, 17 β -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 μ M 5-ASA for 2 hours in the presence of flavonoids at the concentration of 100 μ M. Cellular accumulation percent: (cells/cells plus medium) \times 100. Each value represents the mean \pm SD of four to five experiments.

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

Significant differences from control *P < .05, **P < .01.

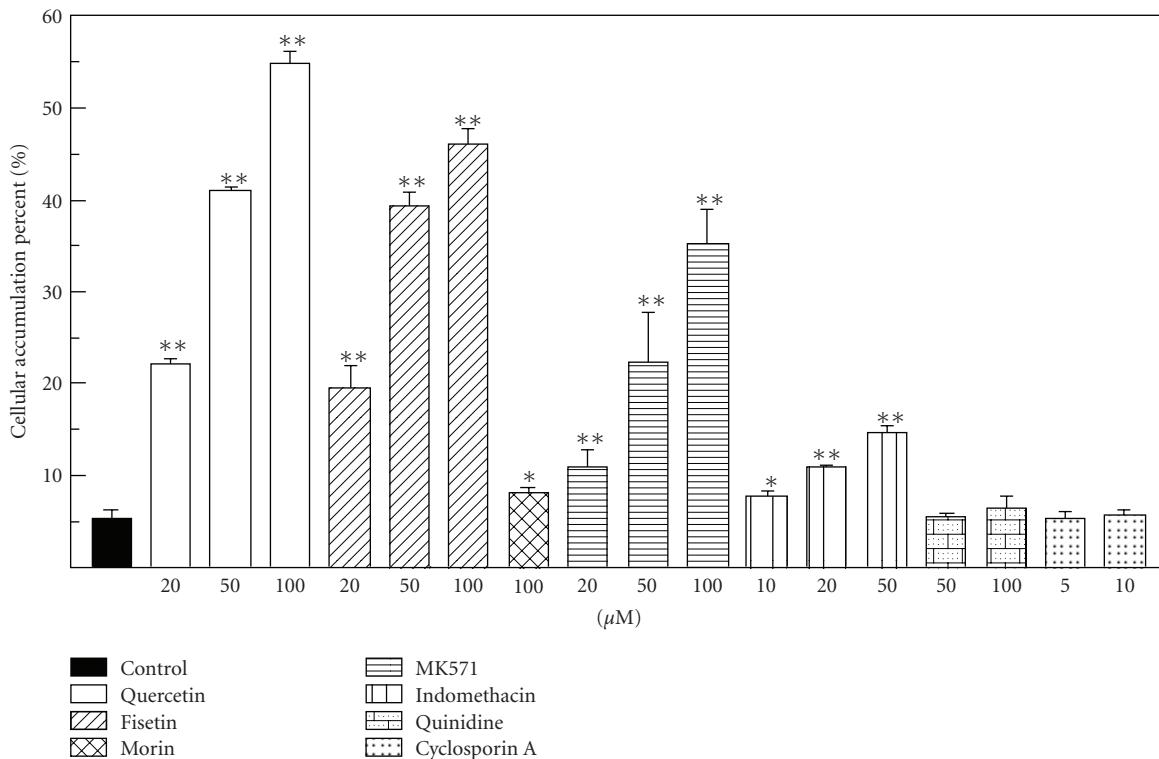


FIGURE 4: The effect of flavonols and transporter inhibitors on the cellular accumulation of *N*-acetyl 5-aminosalicylic acid in Caco-2 cells. Caco-2 cells grown in tissue culture dishes were incubated with 200 μ M 5-ASA for 2 hours in the presence of flavonols and transporter inhibitors at the concentration of 100 μ M. Cellular accumulation percent : (cells/cells plus medium) \times 100. Each bar represents the mean \pm 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

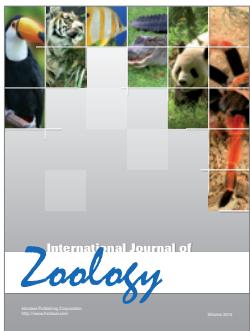
concluded that 1% of the original 4 g dose of quercetin was absorbed [39]. In this study, flavonoids were added at the concentration range from 20 to 100 μ M only into the apical compartment of Caco-2 cells in Transwells that faces to intestinal lumen in vivo. A high luminal level around 100 μ M of flavonoids 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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