Original Article

Luteolin suppresses 5-hydroxytryptamine elevation in stimulated RBL-2H3 cells and experimental colitis mice

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Increased 5-hydroxytryptamine may be associated with the development and progression of inflammatory bowel disease. In this study, we examined the suppressive effect of flavonoids on the increased intra- and extracellular 5-hydroxytryptamine levels in rat mast RBL-2H3 cells, known to produce 5-hydroxytryptamine by the phorbol 12-myristate 13-acetate stimulation. Among the flavonoids examined, luteolin and quercetin significantly reduced the cellular 5-hydroxytryptamine concentration. Gene and protein expression analyses revealed that luteolin significantly suppressed cellular tryptophan hydroxylase 1 expression induced by phorbol 12-myristate 13-acetate stimulation. Mitogen-activated protein kinase/extracellular signal-regulated kinase signaling was also suppressed by luteolin, suggesting that this pathway is one of targets of 5-hydroxytryptamine modulation by luteolin. An *in vivo* experimental colitis model was prepared by administering 2.5% dextran sodium sulfate in drinking water to C57BL/6 mice for seven days. The ingestion of 0.1% dietary luteolin suppressed the increasing 5-hydroxytryptamine in the colorectal mucosa. In conclusion, luteolin possesses a suppressive effect on extensive 5-hydroxytryptamine formation in both experimental RBL-2H3 cells and colitis models.

***Key Words*: 5-hydroxytryptamine, tryptophan hydroxylase 1, RBL-2H3, luteolin, experimental colitis**

Inflammatory bowel disease (IBD), such as Crohn's disease and ulcerative colitis, is one of the chronic inflammatory disorders in patients. IBD patients often suffer from chronic diarrhea, abdominal pain, and melena.[1] These symptoms may disturb work activities and social interactions. Indeed, the quality of life in IBD patients does significantly decrease.[2] Currently, what exactly causes IBD is unknown. However, in recent years it has become clear that immune disorders occur and develop as the disease progresses due to various factors, such as genetics, environment, and intestinal bacteria abnormalities.[3]

5-Hydroxytryptamine (serotonin, 5HT) is an important neurotransmitter that regulates gastrointestinal motility.[4,5] However, excessive 5HT may be associated with IBD pathogenesis and aggravation.[6] Some reports indicate that 5HT producing cells, such as enterochromaffin (EC) cells and mast cells, are increased in IBD patient colons.[7,8] This was also observed in experimental models initiated by chemical administration.[9,10] In addition, 5HT directly or indirectly stimulates immune cells, such as macrophages and T cells.[6,11] It could worsen gut inflammation through enhanced proinflammatory cytokine excretion.[5,12]

It is known that more than 90% of 5HT is generated in the gastrointestinal tract.[13] EC cells predominantly produce 5HT, and mast cells may also contribute to a small degree. Tryptophan hydroxylase (TPH) catalyzes the rate-limiting step in the synthesizes of 5HT from tryptophan.[14] In human TPH exists in two isoforms: TPH-1 and TPH-2.[15] 5HT in the peripheral tissue is synthesized by TPH-1. Secreted 5HT binds 5HT receptors to influence gastrointestinal functions and immunity.[5,16] Secreted 5HT is incorporated into the surrounding epithelial cells by the serotonin transporter (SERT), is degraded to its aldehyde by monoamine oxidase, and metabolically converted to 5-hydroxyindoleacetic acid. Changes in TPH-1 and SERT expression are associated with an increase in 5-HT levels during IBD pathological conditions.[17,18] It has been reported that the polymorphism of the TPH-1 gene affects gastrointestinal symptoms in patients with diarrhea-predominant irritable bowel syndrome, which has some similarities with IBD.[19] The fact suggests that TPH-1 is a key factor for digestive symptoms.

There are some problems with IBD medication with 5-aminosalicylic acid because of undesirable side effects[20,21] or intolerance.[22] As an alternative medicine, various food ingredients can alleviate or prevent colitis.[23] Flavonoids are widely found in plants, fruits, vegetables, and herbs.[24] Flavonoids have broad biological functions such as anti-allergic, anti-inflammatory, and anti-cancer activities.[24] Indeed, flavonoids can improve or prevent IBD in rodent models.[25] For example, rutin ameliorated dextran sodium sulfate (DSS)-induced colitis possibly via the suppression of pro-inflammatory cytokines interleukin-1β (IL-1β) expression.[26] Apigenin supplementation also prevents DSS-induced colitis development by suppressing the nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome.[27] Oral gavage luteolin administration also ameliorated DSS-induced colitis.[28]

As mentioned before, 5HT level modulation is one of the potential therapeutic targets for gut inflammation. However, the inhibitory effect of flavonoids on the 5HT level increase in colitis models remains to be examined. In this study, we compared the flavonoid effect on 5HT concentration modulation using RBL-2H3 cells, widely used in 5HT studies.[29–32] To elucidate the mechanism by which luteolin suppresses the increased intra- and extracellular 5HT concentration, we analyzed TPH-1 and SERT gene and protein expression. We also investigated the

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luteolin's effect on signaling pathways involved in the intra- and extracellular 5HT increase. Furthermore, we confirmed that the luteolin ingestion does suppress the 5HT increase in colitis tissue.

Materials and Methods

Materials. Minimum Essential Media with Earle's salts (MEM) and Chemi-Lumi One L were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). Mammalian protein extraction reagent (M-PER) was purchased from Thermo Fisher Scientific (Waltham, MA). Antibodies were obtained from the following sources: rabbit anti-tryptophan hydroxylase antibody was purchased from Abcam (Cambridge, UK); rabbit anti-SERT (extracellular) antibody was purchased from Allomone Labs (Jerusalem, Israel); rabbit anti-p44/42 MAPK (Erk1/2), rabbit anti-phospho-p44/42 MAPK (Erk1/2), rabbit anti-MEK 1/2, and rabbit anti-phospho-MEK 1/2 antibodies were obtained from Cell Signaling Technology (Danvers, MA); rabbit anti-β-actin antibody was purchased from Medical and Biological Laboratories (Nagoya, Japan); and goat anti-rabbit immunoglobulin G–horseradish peroxidase-conjugated antibody was obtained from DAKO Japan (Tokyo, Japan). Luteolin (98%) for *in vivo* experiments was purchased from Tokyo Chemical Industry (Tokyo, Japan). Luteolin (99%) for *in vitro* experiments, kaempferol, cyanidin chloride, and myricetin were obtained from Extrasynthese (Lyon, France). DSS was obtained from MP Biomedicals (Santa Ana, CA). Apigenin, rutin, quercetin dihydrate, phorbol 12-myristate 13-acetate (PMA), ImmunoStar LD®, PD98059, and ISOGEN I were purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). 5-Hydroxytryptamine-*d*4 (*d*4-5HT) hydrochloride was obtained from Medical Isotope Inc. (Pelham, NH). EzBlock Chemi was purchased from ATTO (Tokyo, Japan).

Cell culture and treatment. RBL-2H3 cells were obtained from the Health Science Research Resources Bank (Osaka, Japan). The cells were maintained in MEM supplemented with 10% fetal bovine serum (FBS), 100 IU/ml penicillin, and 100 μg/ml streptomycin. Typically, the cells were treated with various flavonoids or PD98059 in FBS-free MEM for 30 min. Then, 25 nM PMA was added to each well and further incubated for a range of time. PMA and each flavonoid were dissolved in DMSO, and the final DMSO concentration was 0.12%. To assess cell viability, cells (3.0×10^4 cells) were seeded and cultured in 96-well plates for 24 h. The cells were treated with flavonoids at various concentrations or with 5 μM of the inhibitor in FBS-free MEM medium for 30 min. PMA (25 nM) was added to each well and further incubated for 10 h. After treatment, cell viability was assessed by Cell Count Reagent SF according to the manufacturer's instruction.

Quantitative real-time reverse-transcription. Cells were seeded into 12-well culture plates at a density of 4.5×10^5/well and then cultured overnight. After incubation, the cells were treated as described in the "Cell culture and treatment" section. Total RNA was extracted using ISOGEN I according to the manufacturer's instructions. Reverse transcribed cDNA was prepared using an iScript™ Advanced cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA). cDNA was subjected to quantitative reverse-transcription (qRT)-PCR amplification using MyGo (IT-IS Life Science Ltd, Mahon, Cork, Ireland). The qRT-PCR was performed using MyGo Green Mix Universal (IT-IS Life Science) with previously reported primers for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), TPH-1, and SERT.[32–34] The PCR conditions consisted of 45 cycles with 15 s of denaturation at 95°C, 60 s of annealing, and extension at 60°C. Relative levels of gene expression in each sample were calculated using the comparative $^{\Delta\Delta}$Ct method.

Western blotting. Cells (4.5×10^5/well) were seeded into 12-well culture plates and allowed to attach to the wells overnight. The cells were treated as described in the "Cell culture and treatment section". After the treatments, the cells were washed with ice-cold phosphate-buffered saline (PBS) and lysed using M-PER with protease inhibitor cocktail (Nacalai Tesque, Inc.), phosphatase inhibitor cocktails II, and phosphatase inhibitor cocktails III (Sigma-Aldrich). The samples were centrifuged, and lysate protein concentrations were determined using a BCA protein assay kit. The protein samples were mixed with loading buffer containing 2-mercaptoethanol, separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis using 10% polyacrylamide gels, and electro-transferred on to Immobilon-P Transfer Membranes (Merck-Millipore, MA). The membrane was blocked with EzBlock Chemi for 1 h at room temperature. After washing the membrane three times for 10 min each with 0.1% Tris-buffered saline containing 0.05% Tween-20 (TBST), the membrane was incubated with rabbit polyclonal antibody against TPH-1 (1:500 dilution), SERT (1:500), ERK (1:500), Phospho-ERK (1:500), MEK (1:500), Phospho-MEK (1:500), or β-actin (1:2,000) overnight at 4°C. After washing, the membrane was incubated with horseradish peroxidase-conjugated anti-rabbit immunoglobulin G (1:2,000 for TPH-1, SERT, and ERK, or 1:10,000 for β-actin) in TBST for 1 h at room temperature. The membranes were washed again and protein bands were visualized using ImmunoStar® LD or Chemi-Lumi One L. The image was captured by GeneGnome with GeneSys software ver. 1.6.9.0 (SYNGENE, Bangalore, India).

5HT quantification. Tissue 5HT was measured according to a previous report[35] with some modifications. In brief, the colorectal mucosa (approximately 10 mg) was homogenized in 140 μl ice-cold 0.1% formic acid in water and centrifuged at 14,000 *g* at 4°C for 20 min. The supernatant (75 μl) was mixed with 25 μl internal standard *d*4-5HT (IS, 25 μM). To precipitate proteins, 4 vol. of acetonitrile (CH_3CN) was added to the supernatant and then centrifuged (14,000 *g*, 4°C for 10 min). The supernatant was passed through a solid-phase extraction column (Discovery DSC-18, 500 mg). After washing with 2 ml of methanol/0.1% formic acid in water (5:95, v/v), the supernatant was then eluted with 3 ml of 0.1 M ammonium acetate in methanol. The eluted samples were then evaporated to dryness using vacuum-centrifugation. Finally, the dry residue was reconstituted with a mobile phase liquid (0.1% formic acid in water/CH_3CN [9:1, v/v]) and then centrifuged (10,000 *g*, 4°C for 5 min). The supernatant was then transferred to a vial.

For cell medium (extracellular) samples, cells were treated as described in the "Cell culture and treatment" section, and the cell culture medium (200 μl) was then collected. CH_3CN containing 100 nM IS (800 μl) was added to the medium and centrifuged (14,000 *g*, 4°C for 10 min) for protein removal. For intracellular 5HT, the treated cells were washed by ice-cold PBS followed by lysis with 400 μl of 0.1 M NaOH. The lysate was adjusted to approximately pH 3 by adding HCl. CH_3CN (800 μl) containing 100 nM IS was then added to the lysate (200 μl) and centrifuged. The respective supernatant was then transferred to a vial.

The 5HT contents in the samples were measured by liquid chromatography connected with a quadrupole tandem mass spectrometer [LC/MS/MS (QqQ), a Xevo TQD (Waters, Milford, MA) or API3000 (AB Sciex, Framingham, MA)]. The separation was performed by HPLC [Acquity UPLC H-class (Waters) or Agilent HP1100 (Agilent Technologies, Santa Clara, CA)] with a COSMOSIL PBr (2 × 150 mm, 5 μm; Nacalai Tesque, Inc.) at 0.2 ml/min using 0.1% formic acid in water (solvent A) and CH_3CN (solvent B). The gradient program was as follows: initial B10%, 1 min B10%, 10 min B55%, 14 min B90%, 15 min B10%, and 30 min B10%. Five microliters of the sample were injected. Positive multiple-reaction monitoring

transitions were selected as follows: 5HT 177.1/160.2; IS *d*4-5HT 181.2/164.4.

Animal experiments. The study was approved by the University of Hyogo Ethics Committee (No: 171, 236). A total of 20 6-week-old male C57BL/6N mice were obtained from SLC (Shizuoka, Japan) and housed in controlled conditions [21°C, 50 ± 10% humidity, with a 12-h light/dark cycle (lights on from 9:00 to 21:00)]. After a one-week adaption, mice were randomly divided into three experimental groups: control (n = 5) and DSS groups (n = 10) were fed a standard diet AIN-93G for 14 days. The DSS + LUT groups (n = 5) were fed luteolin (0.1% w/w) with the AIN-93G diet.

After acclimation, the DSS and DSS + LUT mice received distilled drinking water *ad libitum* containing 2.5% (w/v) DSS for 7 days, while the control group was given plain distilled water. The drinking water (with or without DSS) in a bottle was replaced with freshly prepared water once every two days. The body weight of each mouse was recorded every day. Disease activity index (DAI) scores, which were based on body weight loss, stool consistency, and stool bleeding, were calculated as previously described.[36] On day 14, the mice were euthanized under anesthesia using isoflurane, and the blood was immediately collected from the inferior vena cava. The colon length from the proximal colon to the rectum was measured. The colon was washed with ice-cold PBS, the colorectal mucosa was scraped off, and the mucosa was frozen in liquid nitrogen. The samples were stored at −80°C until needed.

Myeloperoxidase (MPO) activity. MPO activity was measured by a method described previously.[37] Briefly, the isolated colon was homogenized in 50 mM hexadecyltrimethylammonium bromide buffer (pH 6.0) at 50 mg wet tissue/ml. After removing debris by centrifugation, the supernatant was incubated with 0.53 mM *o*-dianisidine dihydrochloride and 0.2 mM H_2O_2 in 50 mM potassium phosphate buffer (pH 6.0). The change in absorbance at 450 nm was measured. One MPO activity unit was defined by the consumption of 1 µmol peroxide per minute at 25°C. The activity was expressed as MPO units per mg tissue protein.

Statistical analysis. The animal experiment data shown are representative of two independent experiments. The cell experiments are representative of three independent experiments. Each data was statistically analyzed using Student's t test or one-way ANOVA test with multiple-comparison posthoc analysis (Tukey's or Games-Howell) using SPSS ver. 25.0 (IBM, Armonk, NY) software. A p value <0.05 was considered significant.

Results

Luteolin significantly reduced the intra- and extracellular 5HT levels in RBL-2H3 cells. In our study, the 5HT levels in RBL-2H3 cells were time-dependently increased using PMA as a stimulant. Using this cellular model, we compared seven flavonoids (Fig. 1A) to assess their modulatory effects on 5HT production, excretion, and incorporation. Each flavonoid concentration used for all assays was its maximum concentration that did not cause cytotoxicity. Among the flavonoids used, only luteolin and quercetin significantly suppressed intracellular, extracellular, and total 5-HT levels (Fig. 1B–D). Because

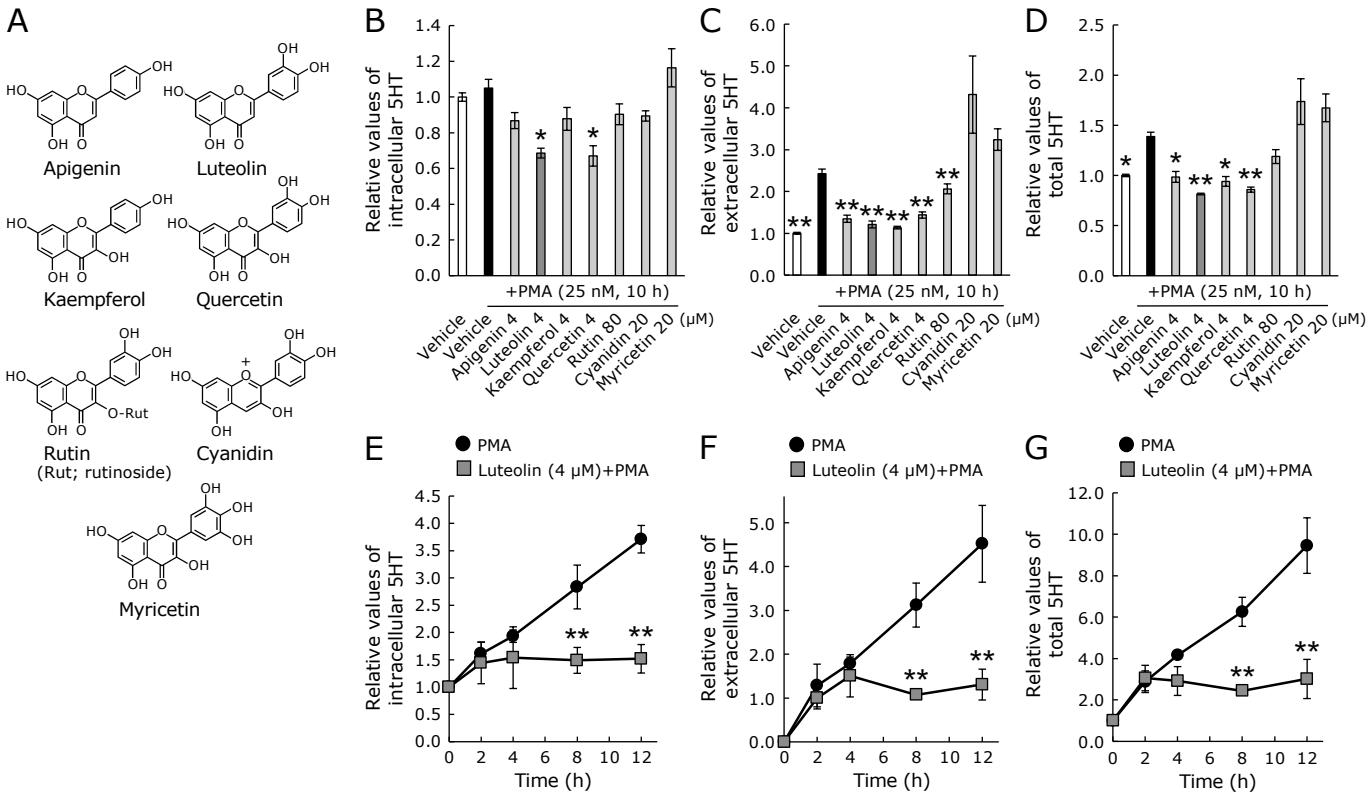

Fig. 1. The flavonoid effects on extracellular and intracellular 5-HT levels in RBL-2H3 cells. Cells were treated with a vehicle or the respective seven flavonoids for (A) 30 min followed by 25 nM PMA stimulation for the indicated time. Intracellular 5HT (B), extracellular 5HT (C), and total 5HT (D) were quantified as described in the Materials and Methods. Time-dependent changes in intracellular, extracellular, and total 5HT levels by luteolin or vehicle are shown in (E–G). Control was 0 h with fresh serum-free medium. The 5HT concentrations were adjusted to the relative control. Data represent the means ± SE. Statistical significance was set at *p<0.05 and **p<0.01 vs vehicle with PMA using Tukey's multiple-comparison posthoc test (B–D) or Student's t test (E–G).

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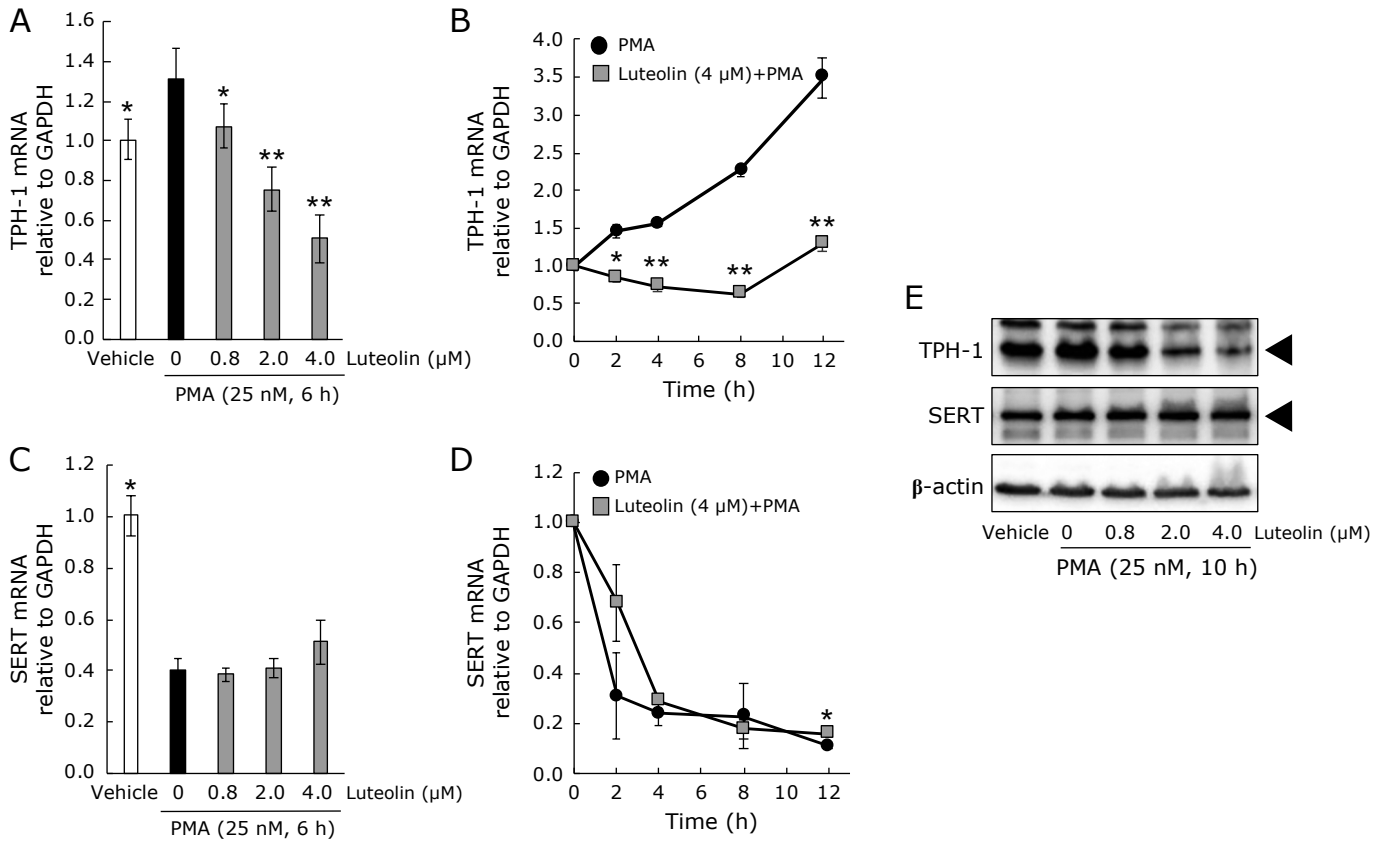

Fig. 2. The luteolin effect on TPH-1 and SERT expression in RBL-2H3 cells. Cells were treated with luteolin (0–4.0 μM) for 0–12 h and then stimulated by PMA for 6 h (for genes) or 10 h (for proteins). The dose-dependent or time-dependent changes of TPH-1 or SERT mRNA expression are shown (A–D). GAPDH mRNA was the internal control. After incubation with PMA, proteins were isolated and probed using Western blotting with anti-TPH-1, anti-SERT, or β-actin antibody (E). Representative results from three independent experiments are shown. The black triangle means the location of the TPH-1 or SERT. Data represent the means ± SE. Statistical significance was set at *p<0.05 and **p<0.01 vs vehicle + PMA using Games-Howell multiple-comparison posthoc test.

luteolin, but not quercetin, suppresses inflammatory symptoms in chemically induced colitis models,[28,38] we focused on luteolin hereafter. Next, the luteolin's effect on 5HT levels was examined. Both intra- and extracellular 5-HT levels were increased by the PMA stimulation in a time-dependent manner. The increases during 8–12 h were extensively suppressed by luteolin treatment (Fig. 1E–G).

The luteolin effect on TPH-1 and SERT expression. To begin to understand the molecular mechanisms of 5HT modulation by luteolin, TPH-1 and SERT expression in RBL-2H3 cells was examined (Fig. 2A–E). A 6-h incubation of luteolin (0–4 μM) dose-dependently suppressed TPH-1 mRNA expression induced by PMA (Fig. 2A). A 12-h incubation (4 μM) completely suppressed the PMA-induced TPH-1 mRNA expression (Fig. 2B). The PMA-induced TPH-1 protein upregulation was also suppressed by luteolin in a dose-dependent manner (Fig. 2E). The PMA treatment downregulated SERT mRNA but not SERT protein (Fig. 2C–E). Luteolin had no significant effect on SERT expression, except the mRNA expression after 12 h PMA stimulation (Fig. 2C–E).

The MEK/ERK signaling pathway effect on 5HT production. Previous studies reported that extracellular signal-related kinase (ERK) phosphorylation affects 5HT generation and secretion from neoplastic EC cells (KRJ-I).[39,40] Therefore, we confirmed whether 5HT production responds to the MEK/ERK signaling pathway in the PMA-stimulated cells using a specific MAPK/ERK kinase (MEK) inhibitor, PD98059. The extracellular and total 5HT levels were significantly decreased by PD98059, while the intracellular 5HT concentrations did not change (Supplemental Fig. 1A–C*). PD98059 also suppressed TPH-1 mRNA and protein expression (Supplemental Fig. 1D and E*). We then investigated the luteolin effect on MEK and ERK phosphorylation levels (Fig. 3). The phosphorylated MEK (Pi-MEK1/2) and ERK (Pi-ERK1/2) levels increased after PMA stimulation, and the ERK phosphorylation tended to decrease following luteolin treatment.

Luteolin attenuated DSS-induced colitis and reduced the elevated 5HT levels in colorectal mucosa. To examine the *in vivo* luteolin effect on 5HT modulation, the inflammation extent in a DSS-induced colitis murine model was first estimated by evaluating the body weight alteration rate, colon length shortening, and DAI score (Fig. 4A). Neutrophil infiltration into the colorectal mucosa was also measured by MPO activity. Before the experiment, we confirmed that a luteolin diet without DSS did not affect body weight and other indexes (unpublished observation). The body weight of 2.5% DSS-treated mice decreased significantly from day-4 compared with control. DSS + LUT (0.1% luteolin and DSS) mice also decreased in weight (Fig. 4B). The luteolin diet improved the DAI score and partly prevented the colon length shortening induced by DSS for seven days (Fig. 4C and D). Luteolin supplementation attenuated the increasing rate of MPO activity by DSS challenge (Fig. 4E). The increased 5HT level in DSS-induced colitis mice mucosa was also significantly reduced by the luteolin diet (p<0.05, Fig. 4F).

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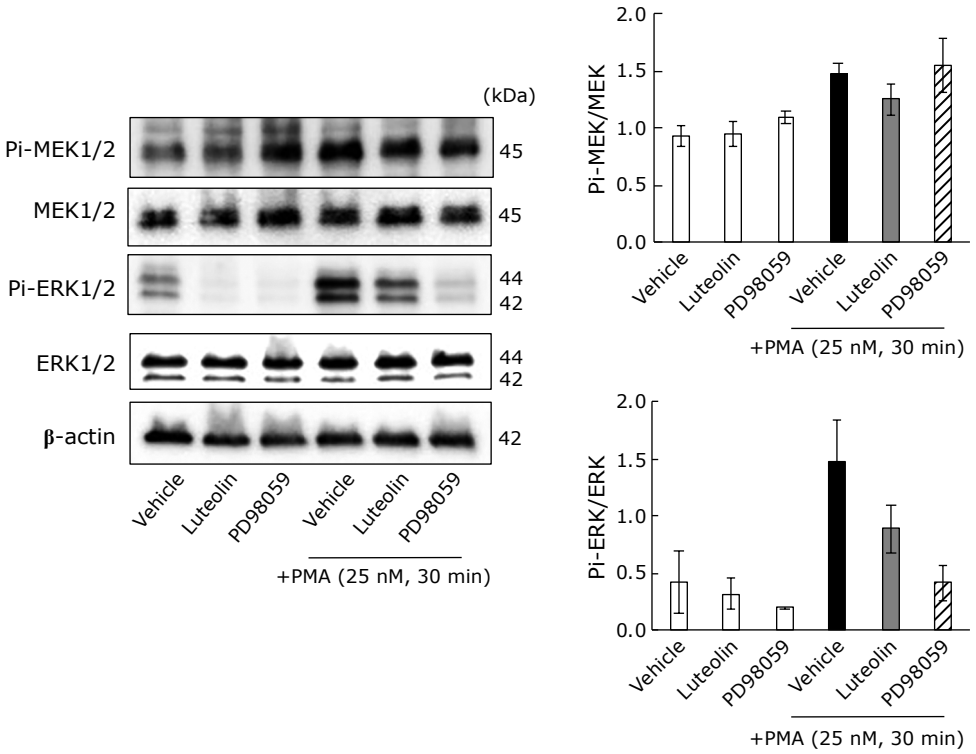

Fig. 3. The luteolin suppresses the MEK/ERK signal pathway which may contribute to 5HT increase in RBL-2H3 cells by PMA stimulation. Cells were treated with luteolin (4 μM) or PD98059 (5 μM) for 30 min and then stimulated by PMA for 30 min. Proteins were isolated, separated, and blotted with anti-phosphorylated MEK, anti-MEK, anti-phosphorylated ERK, anti-ERK, or anti-β-actin antibody. The relative semiquantitative analysis was based on optical density by ImageJ software, ver. 1.41 (National Institutes of Health; Bethesda, MD).

Discussion

In this study, we found that luteolin can suppress the increase in 5HT and TPH-1 expression in PMA-stimulated RBL-2H3 cells. Luteolin also showed the suppressive effects on 5HT increment in DSS-challenged mice colorectal mucosa. Firstly, we evaluated the effect of seven flavonoids on 5HT modulation using the cultured cells. Among them, the catechol-type flavonoids except cyanidin (anthocyanidin), luteolin and quercetin, suppressed both intra- and extracellular 5HT increases (Fig. 1B and C). The detailed structure-activity relationship for 5HT modulation remains to be elucidated. Since luteolin suppresses chemical-induced intestinal inflammation,[28,38] luteolin was selected for further study.

Multiple mechanisms for the increase in 5HT under inflammatory conditions might be underlying *in vivo* and *in vitro*. TPH-1 knockouts delay onset of inflammatory disease and decrease its severity in the DSS-induced colitis model.[41] Blocking peripheral 5HT synthesis by a TPH inhibitor reduces the severity of chemical-induced intestinal inflammation.[42] The SERT expression level is considered to be another important determinant of the extracellular 5HT amount. It has been reported that ulcerative colitis patients and chemical-induced colitis mice reduce SERT immunoreactivity and/or mRNA expression.[43,44] SERT knockout mice have significantly worse intestinal inflammation.[45] Then we focused on the TPH-1 and SERT expression changes from key regulators for intra- and extracellular 5HT levels when luteolin was supplemented in RBL-2H3 cells. Luteolin suppressed the increased TPH-1 mRNA and protein expression stimulated by PMA (Fig. 2A, B, and E), suggesting that TPH-1 is one of the critical molecules targeted by luteolin. As far as we know, there is no report on TPH-1 suppression affected by a flavonoid. By contrast, luteolin did not modulate SERT expression (Fig. 2D and E). Previous studies showed that luteolin activates SERT in rat SERT-overexpressing Chinese hamster ovary cells.[46] To estimate the influence of luteolin on SERT-dependent 5HT production, it will be necessary to investigate the SERT expression on the cell membrane and also its activity in RBL-2H3 cells.

The increased ERK phosphorylation may be involved in 5HT synthesis and release in KRJ cells.[39] Pretreating RAW 264.7 cells with luteolin inhibited LPS-induced ERK1/2 phosphorylation.[47] Indeed, luteolin suppressed MEK and ERK phosphorylation in RBL-2H3 cells stimulated by PMA (Fig. 3). In addition to the MEK-ERK signal, luteolin affects various molecular signals, such as ion channels, receptors, and other signaling pathways,[48,49] possibly resulting in suppressing intra- and extracellular 5HT levels. Therefore, an advanced study is required to elucidate the precise molecular mechanism of 5HT suppression by luteolin.

Concerning dietary luteolin *in vivo*, DSS-treated mice increased 5HT levels in colon mucosa approximately 2.3-fold higher than compared with control mice, along with increased DAI score and other biochemical indexes (Fig. 4C–F). The luteolin diet (0.1%) partially improved colitis symptoms, including DAI score and colon length shortening. Luteolin supplementation improves colitis by oral administration,[28,38,50] and dietary supplementation with 0.1% luteolin is also effective in mice. Besides, we revealed that the 5HT level increased by DSS treatment was significantly suppressed by the luteolin diet (Fig. 4F). It is noteworthy that the 5HT increase in the DSS colitis model may be due to the increased EC cells and TPH-1 expression, as well as increased interleukin 13 and intestinal flora.[51,52] To the best of our knowledge, this is the first report

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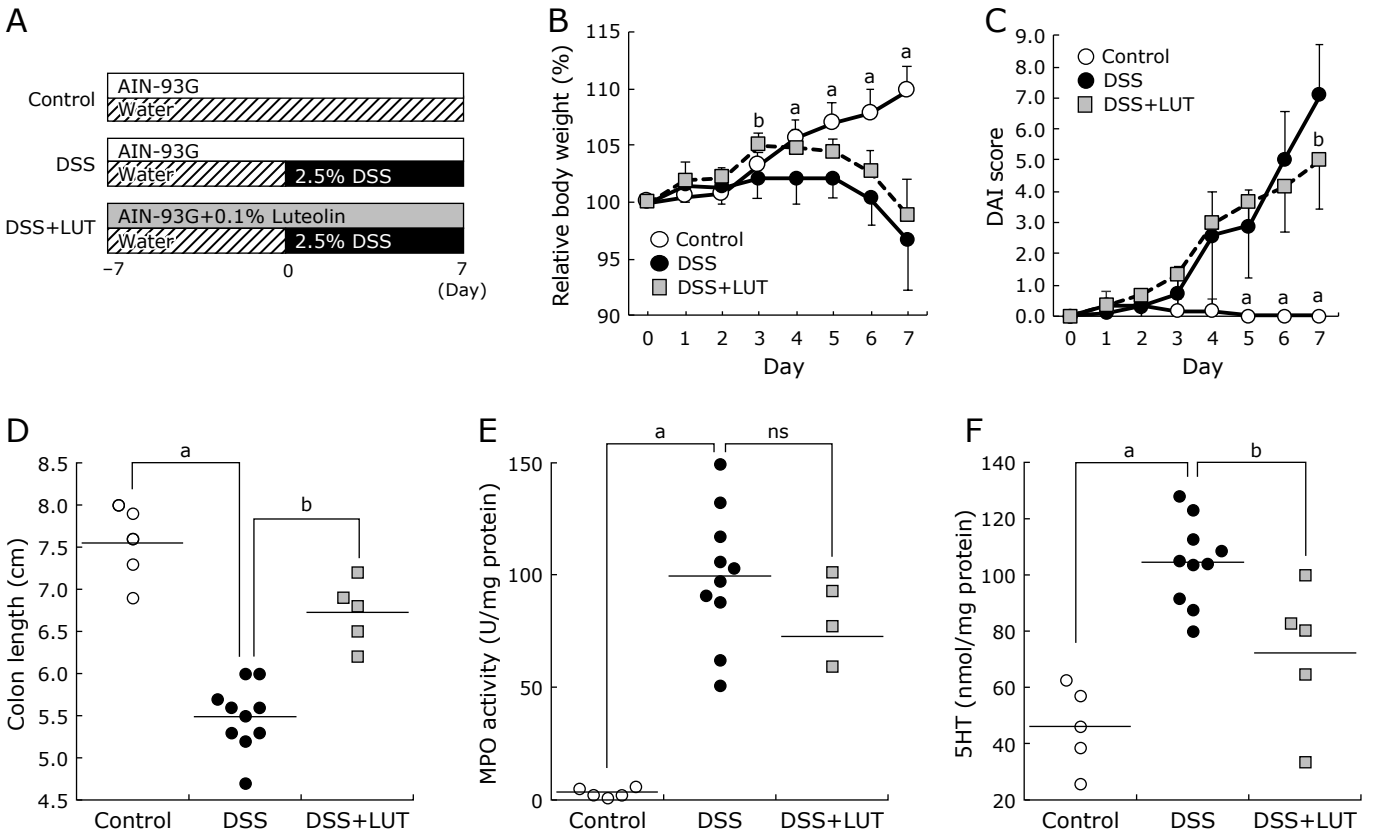

Fig. 4. The dietary luteolin effect on disease manifestations, myeloperoxidase activity, and mucosal 5HT in DSS-induced colitis mice. (A) Experimental design of DSS colitis induction with control or luteolin diet. The animals were split into three groups: control, DSS, and DSS + LUT groups as described in the "Materials and Methods" section. Seven-week old mice were fed with a standard diet (the control or DSS group) or received a diet supplemented with 0.1% luteolin (DSS + LUT group). Colitis was induced by administering 2.5% DSS in drinking water for both DSS and DSS + LUT groups. (B) The body weight change rate. (C) Disease activity index (DAI) score. (D) Colon length. (E) Myeloperoxidase (MPO) activity in colorectal tissue. (F) 5HT contents in colorectal mucosa. Control: open circle, DSS: closed circle, DSS + LUT: light gray square. Groups were compared by Tukey's or Games-Howell multiple-comparison posthoc test (n = 5 mice for control and DSS + LUT groups, n = 10 mice for DSS group). Data represent the means ± SE. Significance was compared with the DSS treatment group. Values designated by different letters (a, b) are significantly different (p<0.05).

that luteolin intake suppressed mucosal 5HT in a colitis model. In contrast, the current study could not provide the outcome of *in vivo* modulation of TPH-1 expression by dietary luteolin; it is a remaining task to be determined. Furthermore, the changes in SERT expression should be evaluated to understand the regulatory mechanism of the local 5HT level in near future.

In conclusion, this study revealed that luteolin reduces the 5HT levels, at least in part, by inhibiting TPH-1 in RBL-2H3 cells. This mechanism may also be applicable for *in vivo* 5HT suppression by luteolin administration. However, this is just the start of uncovering the full mechanism. Dietary luteolin may have the potential to ameliorate inflammatory conditions by decreasing overproduced 5HT.

Author Contributions

NS planned and performed all experiments, and wrote the draft of this paper. AM and YN discussed the data and helped to write this paper. HA and TN advised and supported this study. YK supervised, wrote, and edited the paper.

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Conflict of Interest

No potential conflicts of interest were disclosed.

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