

Potent Suppressive Effects of 3-O-Methylquercetin 5,7,3',4'-O-Tetraacetate on Ovalbumin-Induced Airway Hyperresponsiveness

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Abstract

We investigated the suppressive effects of 3-O-methylquercetin 5,7,3',4'-O-tetraacetate (QMTA), a more-potent phosphodiesterase (PDE)3/4 inhibitor than quercetin 3-O-methyl ether (3-MQ), which has been reported to have the potential for treating asthma, against ovalbumin (OVA)-induced airway hyperresponsiveness (AHR). The IC₅₀ value of QMTA for PDE3 was significantly less than that for PDE4. According to the Lineweaver-Burk analysis, QMTA (1–10 μ M) competitively inhibited PDE3 and PDE4 activities. The K_i values were 0.9 ± 0.3 ($n = 5$) and 3.9 ± 0.5 ($n = 5$) μ M, respectively, which significantly differed from each other, suggesting that QMTA has higher affinity for PDE3 than for PDE4. QMTA (3–10 μ M) concentration-dependently relaxed the baseline level, and significantly inhibited cumulative OVA (10–100 μ g/mL)-induced contractions in isolated sensitized guinea pig trachealis suggesting that QMTA has bronchodilator and inhibiting effects on mast cell degranulation. After the secondary challenge, the AHR was measured in unrestrained OVA-sensitized mice, with nebulized methacholine (MCh, 6.25–50 mg/mL), by barometric plethysmography using a whole-body plethysmograph. In the present results, QMTA (3–10 μ mol/kg, *i.p.*) dose-dependently attenuated the enhanced pause (P_{enh}) value induced by MCh (25–50 mg/mL). QMTA (3–10 μ mol/kg, *i.p.*) also significantly inhibited total inflammatory cells, macrophages, neutrophils, lymphocytes, and eosinophils in BALF after de-

termination of P_{enh} values. It also significantly suppressed the release of interleukin (IL)-2, IL-4, IL-5, IFN- γ , and TNF- α , with the exception that 3 μ mol/kg QMTA did not suppress the releases of IL-5. QMTA even at 1 μ mol/kg significantly inhibited eosinophils, IL-2, and TNF- α . In conclusion, our results strongly suggest that QMTA has greater potential than 3-MQ for the treatment of asthma.

Key words

3-O-methylquercetin 5,7,3',4'-O-tetraacetate · phosphodiesterase inhibitor · ovalbumin · inflammatory cells · cytokines · asthma

Abbreviations

AHR: airway hyperresponsiveness
BALF: bronchoalveolar lavage fluid
CBA: cytometric bead array
IFN- γ : interferon- γ
IL: interleukin
OVA: ovalbumin
PDE: phosphodiesterase
P_{enh}: enhanced pause
QMTA: 3-O-methylquercetin 5,7,3',4'-O-tetraacetate
TNF- α : tumor necrosis factor- α

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Bibliography

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Introduction

Phosphodiesterases (PDEs) have been classified according to their primary protein and cDNA sequences, co-factor and substrate specificities, and pharmacological roles. It is now known that PDEs comprise at least 11 distinct enzyme families that hydrolyze adenosine 3',5'-cyclic monophosphate (cAMP) and/or guanosine 3',5'-cyclic monophosphate (cGMP) [1]. PDE1–5 isozymes, which are calcium/calmodulin-dependent (PDE1), cGMP-stimulated (PDE2), cGMP-inhibited (PDE3), cAMP-specific (PDE4), and cGMP-specific (PDE5), have been found to be present in the human bronchi [2], canine trachea [3], guinea pig lung [4]. PDE3, PDE4, or dual PDE3/4 inhibitors were reported to have potential for use in the treatment of asthma [4].

Quercetin 3-*O*-methyl ether (3-MQ) is a major constituent of *Rhamnus nakaharai* Hayata (Rhamnaceae), which has been used as a folk medicine in Taiwan for treating constipation, inflammation, tumors, and asthma [5]. 3-MQ was reported to exhibit dual inhibition of PDE3/4 (with IC_{50} values of 1.6 μ M and 28.5 μ M, respectively) in our laboratory [6]. Recently, 3-MQ was reported to exert its anti-inflammatory effect through the inhibition of iNOS DNA transcription [7], and suppress airway hyperresponsiveness (AHR) [8]. Therefore, we were interested in investigating the suppressive effects of 3-*O*-methylquercetin 5,7,3',4'-*O*-tetraacetate (QMTA; Fig. 1), a more-potent PDE3/4 inhibitor than 3-MQ, on ovalbumin (OVA)-induced airway hyperresponsiveness. The rationale of our proposal was based on the preliminary observation that quercetin 3,5,7,3',4'-pentaacetate was more selective and potent than quercetin in the inhibition of PDE1–5.

Materials and Methods

Reagents and drugs

According to the method described by Ferte et al. [9], QMTA (Fig. 1) was synthesized by acetylation from 3-MQ which was isolated from *R. nakaharai* [10], and identified by spectral methods, including UV, IR, MS, and NMR techniques (spectral data of QMTA are obtainable on request from the author of correspondence). The purity of QMTA was 98%. Methacholine (MCh), indomethacin, nifedipine, ovalbumin (OVA), dimethyl sulfoxide (DMSO), Tris-HCl, bis-Tris, *dl*-dithiothreitol, ethylenediaminetetraacetic acid (EDTA), bovine serum albumin (BSA), cAMP, cGMP, calmodulin, Dowex resin, and *Crotalus atrox* snake venom

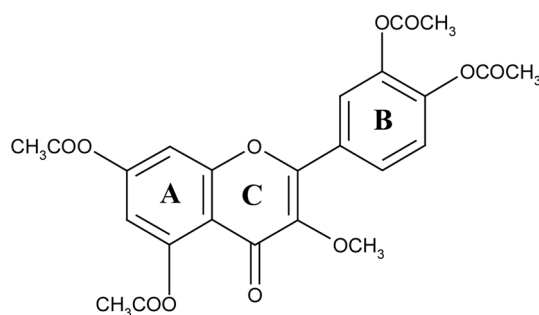


Fig. 1 Chemical structure of 3-*O*-methylquercetin 5,7,3',4'-*O*-tetraacetate (QMTA, M.W. 484.23).

were purchased from Sigma Chemical (St. Louis, MO, USA). Vinpocetin, *erythro*-9-(2-hydroxy-3-nonyl) adenine HCl (EHNA), milrinone, rolipram, 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20–1724), and zaprinast were purchased from Biomol (Plymouth Meeting, PA, USA). [^3H]-cAMP and [^3H]-cGMP were purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). Freund's complete adjuvant was purchased from Pierce Biotechnology (Rockford, IL, USA). Other reagents, such as $CaCl_2$, $MgCl_2$, and NaCl, were of analytical grade. Ro 20–1724 and rolipram were dissolved in a mixture of ethyl alcohol and DMSO (1:1). Ro 20–1724 and EHNA were dissolved in ethyl alcohol. QMTA and milrinone were dissolved in DMSO. EDTA was dissolved in 3 N NaOH. Other drugs were dissolved in distilled water. The final concentration of ethyl alcohol or DMSO was $\leq 0.1\%$, and did not significantly affect the activities of PDE isozymes.

Airway hyperresponsiveness *in vivo*

Under a protocol approved by the Animal Care and Use Committee of Taipei Medical University, the following *in vivo* and *in vitro* experiments were performed. Female BALB/c mice at 8 to 12 week of age were obtained from the National Laboratory Animal Center (Taipei, Taiwan). The mice were housed in ordinary cages at $22 \pm 1^\circ C$ with a humidity of 50–60% under a constant 12/12-h light/dark cycle and provided with OVA-free food and water *ad libitum*. Six mice in each group were sensitized with OVA by an intraperitoneal (*i.p.*) injection of 20 μ g of OVA emulsified in 2.25 mg aluminum hydroxide gel in a total volume of 100 μ L on days 0 and 14. A sham (non-sensitized) group of mice received saline instead of OVA was used for comparison. Mice were challenged with 1% OVA in saline for 30 min on days 28, 29, and 30 by ultrasonic nebulization via the airway. Six weeks after the last of the three primary OVA challenges, mice were exposed to 1% OVA for 30 min by nebulization as a secondary challenge [11]. AHR was assessed on day 74 (48 h after 1% OVA provocation) in each group. Each group of mice was intraperitoneally injected with the vehicle (control), or QMTA (1–10 μ mol/kg) 2 h before and 6 and 24 h after the OVA provocation. The vehicle, a mixture of alcohol:polyethyleneglycol 400:saline (1:14.5:14.5), or QMTA was injected at a volume of 0.01 mL/g of body weight. The AHR was measured in unrestrained animals by barometric plethysmography [12] using a whole-body plethysmograph (WBP) and analyzed using software of Life Science Suite P3 Analysis Modules (Gould, LDS Test and Measurement LLC; Valley View, OH, USA). Mice were placed in the main chamber of the WBP, and enhanced pause (P_{enh}) values were determined by readings of breathing parameters for 3 min before (baseline) and after nebulization of a phosphate-buffered solution (PBS). Then the P_{enh} values were determined by same method with increasing doses (6.25–50 mg/mL) of methacholine (MCh) for 3 min for each nebulization. Twenty-four hours after the P_{enh} determination, these mice were anesthetized with pentobarbital (50 mg/kg, *i.p.*), and lavaged via a tracheal tube with PBS (1 $\times$ 1.0 mL, $37^\circ C$). The collected bronchoalveolar lavage fluid (BALF) was centrifuged at 500 rpm for 5 min, and the pellet was resuspended in ACK lysing buffer to lyse the residual erythrocytes in each sample. The number of inflammatory cells was counted using a hemacytometer (Hausser Scientific; Horsham, PA, USA). Cytospin slides were stained and differentiated in a blinded fashion by counting at least 100 cells under the light microscope. After centrifugation, the supernatant was stored at $-20^\circ C$ until determi-

nation of cytokines, including interleukin (IL)-2, IL-4, IL-5, tumor necrosis factor (TNF)- α , and interferon (IFN)- γ by flow cytometric methods [13] using mouse Th1/Th2 cytokine CBA kits (Pharmingen; San Diego, CA, USA) according to the recommendations of the manufacturer.

Airway hyperresponsiveness *in vitro*

Male Hartley guinea pigs (500–600 g), obtained from the National Laboratory Animal Center (Taipei, Taiwan), were sensitized by intramuscular injections of 0.7 mL of 5% (w/v) OVA in saline on days 1, 4, and 43, and in adjuvant on days 25 and 39 into each thigh, respectively, according to the method described by Underwood et al. [4] and modified by us. Three days after the last injection, the sensitized guinea pigs were sacrificed by cervical dislocation, and the tracheas were removed. Each trachea was cut into six segments. Each segment consisted of three cartilage rings. All segments were cut open opposite the trachealis. After the segments were randomized to minimize regional variability, they were tied at one end to holders via silk sutures, placed in 5 mL of normal Krebs solution containing indomethacin (3 μ M), gassed with a 95% O₂ plus 5% CO₂ mixture at 37 °C, and attached by the other end of each segment to force displacement transducers (Grass FT03; Grass; Quincy, MA, USA) for the isometric recording of tension changes on a polygraph (Gould RS3200; Gould). The composition of the normal Krebs solution was (mM): NaCl 118, KCl 4.7, MgSO₄ 1.2, K₂PO₄ 1.2, CaCl₂ 2.5, NaHCO₃ 25, and dextrose 10.1. The tissues were suspended in normal Krebs solution under an initial tension of 1.5 g and allowed to equilibrate for at least 1 h with washing at 15-min intervals. After the tissues were precontracted with KCl (60 mM) and washed with normal Krebs solution, OVA (0.1–100 μ g/mL) was cumulatively added, and contractions were allowed to reach a steady state at each concentration. To evaluate the suppressive effect of QMTA on OVA-induced contractions, each tissue was preincubated with each concentration (1, 3, or 10 μ M) of QMTA or its vehicle for 15 min and then challenged with cumulative OVA again. Therefore, the log concentration-response curves of OVA were constructed in the absence and presence of QMTA. The tension of the precontraction induced by KCl was set as 100%.

Inhibition on PDE activities and the Lineweaver-Burk analysis

The activities of PDE1–5, partially separated from guinea pig lungs and hearts according to the method described by Ko et al. [6], were measured with a two-step procedure according to the method of Thompson and Appleman [14], using cAMP with [³H]-cAMP or cGMP with [³H]-cGMP as substrates. The enzyme preparation (25 μ L) was incubated for 30 min at 37 °C in a total assay volume of 100 μ L containing 50 mM Tris-HCl (pH 7.4), 3 mM MgCl₂, 1 mM dithiothreitol, 0.05% BSA, 1 μ M cAMP with 0.2 μ Ci [³H]-cAMP as a substrate alone or in the presence of 0.1 units calmodulin with 10 μ M CaCl₂ or 5 μ M cGMP, and 1 μ M cGMP with 0.2 μ Ci [³H]-cGMP as another substrate alone or in the presence of 0.1 units calmodulin with 10 μ M CaCl₂. In tests of enzyme inhibition, the reaction mixture contained 10 μ L of vehicle or inhibitors, at various concentrations of QMTA derivatives or selective PDE1–5 inhibitors, such as vinpocetin [15], EHNA [16], milrinone [17], Ro 20–1724 [18], and zaprinast [19] as reference drugs, respectively. The reagents and homogenate were mixed on ice, and the reaction was initiated by transferring the mixture to a water bath at 37 °C. Following 30-min incubation,

the reaction was stopped by transferring the reaction vessel to a bath of boiling water for 3 min. After cooling on ice, 20 μ L of a 1 mg/mL solution of *Crotalus atrox* venom was added to the reaction mixture, and the mixture was incubated at 37 °C for 10 min. Unreacted [³H]-cAMP or [³H]-cGMP was removed by the addition of 500 μ L of a 1-in-1 Tris-HCl (40 mM) buffer suspension of Dowex resin (1 $\times$ 8–200) with incubation on ice for 30 min. Each tube was then centrifuged for 2 min at 6000 rpm, and 150 μ L of the supernatant were removed for liquid scintillation counting. Less than 10% of the tritiated cyclic nucleotide was hydrolyzed in this assay. The total protein in each fraction used was assayed according to the method described by Bradford [20]. The PDE activities are shown as nmol/mg/min in the Lineweaver-Burk analysis.

Statistical analysis

The IC₅₀ values of QMTA for PDE1–5 were calculated using a non-linear regression analysis by the software SigmaPlot 10.0 (Sigma Chemical; St. Louis, MO, USA), with the exception that that for PDE3 was calculated by extrapolation. All values are given as the mean $\pm$ SEM. Differences among values were statistically calculated by one-way analysis of variance (ANOVA), and then determined by Dunnett's test. The difference between two values, however, was determined by Student's *t*-test. Differences were considered statistically significant if the *p* value was < 0.05.

Results

The P_{enh} values at the baseline for non-sensitized, control (vehicle), and 1, 3, and 10 μ mol/kg (*i.p.*) QMTA administered groups were 4.40 $\pm$ 0.66, 4.14 $\pm$ 0.47, 3.61 $\pm$ 0.26, 3.46 $\pm$ 0.50, and 3.93 $\pm$ 0.29, respectively, and these values did not significantly differ from each other. The P_{enh} values of PBS nebulization for each group were 4.06 $\pm$ 0.63, 4.01 $\pm$ 0.49, 3.63 $\pm$ 0.65, 3.60 $\pm$ 0.30, and 3.78 $\pm$ 0.61, respectively, which also did not significantly differ from each other. Administration of nebulized PBS did not affect the P_{enh} value of each baseline group. However, MCh (6.25–50 mg/mL) concentration-dependently increased P_{enh} values from 1.18 $\pm$ 0.05-fold of PBS exposure to 2.90 $\pm$ 0.20-fold in control sensitized and challenged mice (Fig. 2A). The P_{enh} value of MCh (50 mg/mL) in the control sensitized and challenged mice was significantly greater than that (2.21 $\pm$ 0.09-fold) in non-sensitized but challenged (non-sensitized) mice (Fig. 2A). QMTA (3–10 μ mol/kg, *i.p.*) dose-dependently and significantly attenuated the enhancement of the P_{enh} value induced by MCh at 25–50 mg/mL, as did QMTA (10 μ mol/kg, *i.p.*) even at 12.5 mg/mL MCh (Fig. 2A). The total inflammatory cells, macrophages, lymphocytes, neutrophils, and eosinophils from the BALF of control sensitized and challenged mice significantly increased when compared to non-sensitized mice (Fig. 2B). QMTA (3–10 μ mol/kg, *i.p.*) also significantly suppressed the increase in total inflammatory cells, macrophages, lymphocytes, neutrophils, and eosinophils, as did QMTA at 1 μ mol/kg (*i.p.*) in eosinophils (Fig. 2B). IL-2, IL-4, IL-5, TNF- α , and IFN- γ from the BALF of control sensitized and challenged mice significantly increased when compared to non-sensitized mice (Fig. 2C). QMTA (3–10 μ mol/kg, *i.p.*) also significantly suppressed the increases in these cytokines, with the exception of IL-5 at a dose of 3 μ mol/kg (Fig. 2C). How-

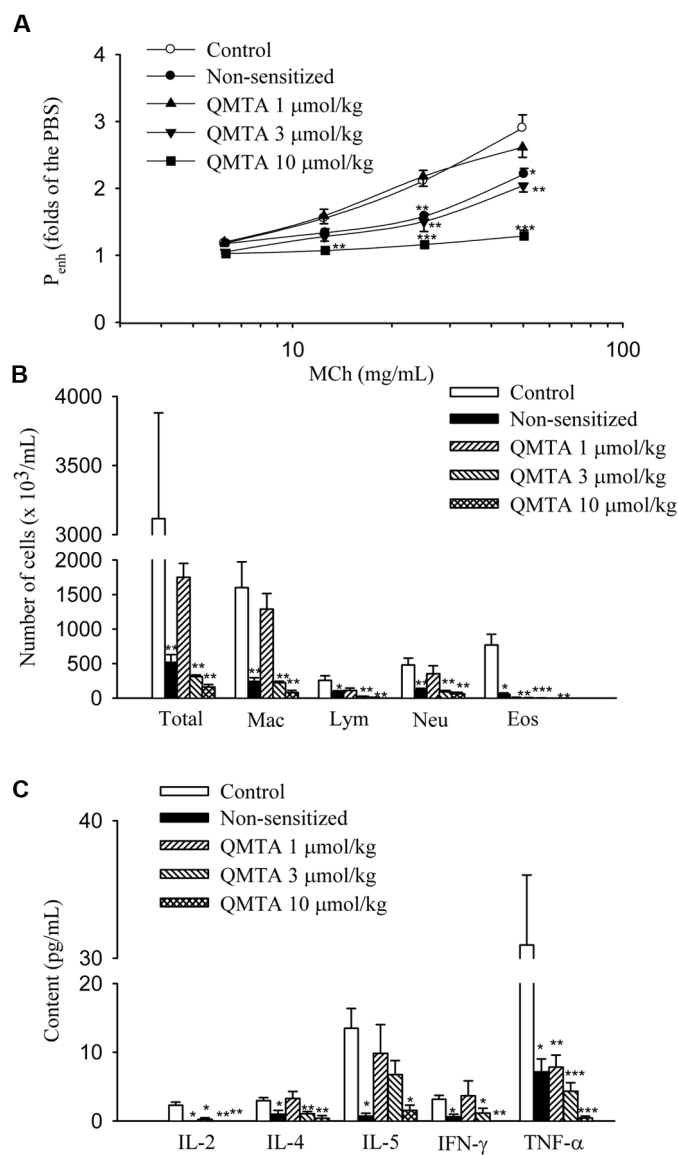


Fig. 2 Effects of 3-O-methylquercetin 5,7,3',4'-O-tetraacetate (QMTA) (1–10 μ mol/kg, *i.p.*) on the enhanced pause (P_{enh}) (A), inflammatory cells (B), and cytokines (C) in sensitized mice which received aerosolized methacholine (MCh, 6.25–50 mg/mL) 2 days after secondary allergen challenge. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when compared with the vehicle (control). The number in each group of mice was 6. Total = total cells; Mac = macrophages; Lym = lymphocytes; Neu = neutrophils; Eos = eosinophils.

ever, QMTA at the smallest dose (1 μ mol/kg, *i.p.*) even significantly suppressed the increases in IL-2 and TNF- α (Fig. 2C).

In isolated sensitized guinea pig trachea, 60 mM KCl evoked a contraction and increased tension to 0.59 ± 0.09 g ($n = 5$), which was set as 100%. OVA (0.01–100 μ g/mL) alone concentration-dependently enhanced tension from the baseline to $90.5 \pm 16.8\%$ ($n = 5$) or $111.1 \pm 7.0\%$ ($n = 5$) of the 60 mM KCl-induced contraction, respectively (Fig. 3A, B). The log concentration-response curve of OVA was unaltered by 1 μ M nifedipine, a selective voltage-dependent calcium channel blocker [21] (data not shown). The vehicle of QMTA or Ro 20–1724 did not affect OVA (100 μ g/mL)-induced maximal contractions. However, QMTA (1,

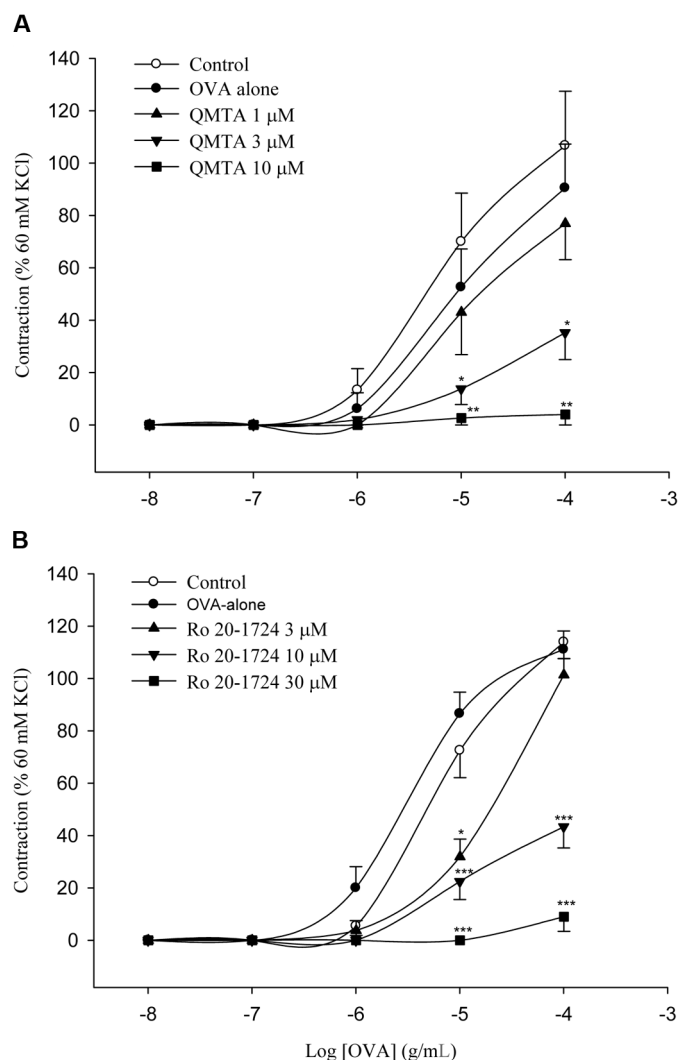


Fig. 3 Effects of 3-O-methylquercetin 5,7,3',4'-O-tetraacetate (QMTA) (A) and Ro 20–1724 (B) on cumulative ovalbumin (OVA)-induced contractions in isolated sensitized guinea pig trachealis. The number of experiments was 5. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ when compared with the vehicle (control).

3 and 10 μ M) concentration-dependently relaxed 0.02 ± 0.02 g ($n = 5$), 0.08 ± 0.04 g ($n = 5$) and 0.15 ± 0.06 g ($n = 5$) of the resting tension, respectively. QMTA at 10 μ M exerted a significant relaxation of the resting tension. Nifedipine at 1 μ M also significantly relaxed 0.24 ± 0.08 g ($n = 3$) of the resting tension. QMTA (3–10 μ M) significantly inhibited OVA (10–100 μ g/mL)-induced contractions, when compared to the vehicle (Fig. 3A). The same was true for Ro 20–1724 (3–30 μ M) with the exception that Ro 20–1724 (3 μ M) did not inhibit the contraction induced by OVA at 100 μ g/mL (Fig. 3B).

QMTA (1–100 μ M) concentration-dependently inhibited PDE1–5 activities (Fig. 4), with respective IC_{50} values of 3.1 ± 0.5 ($n = 4$), 8.5 ± 0.2 ($n = 3$), 0.8 ± 0.5 ($n = 3$), 3.9 ± 0.6 ($n = 3$), and 11.1 ± 1.6 ($n = 3$) μ M. The IC_{50} value of QMTA for PDE4 was significantly greater than that for PDE3, but less than those for PDE2 and PDE5, although it did not differ from that for PDE1. However, the IC_{50} value of QMTA for PDE4 was significantly less than that of 3-MQ for the same previously reported isozyme [6]. According

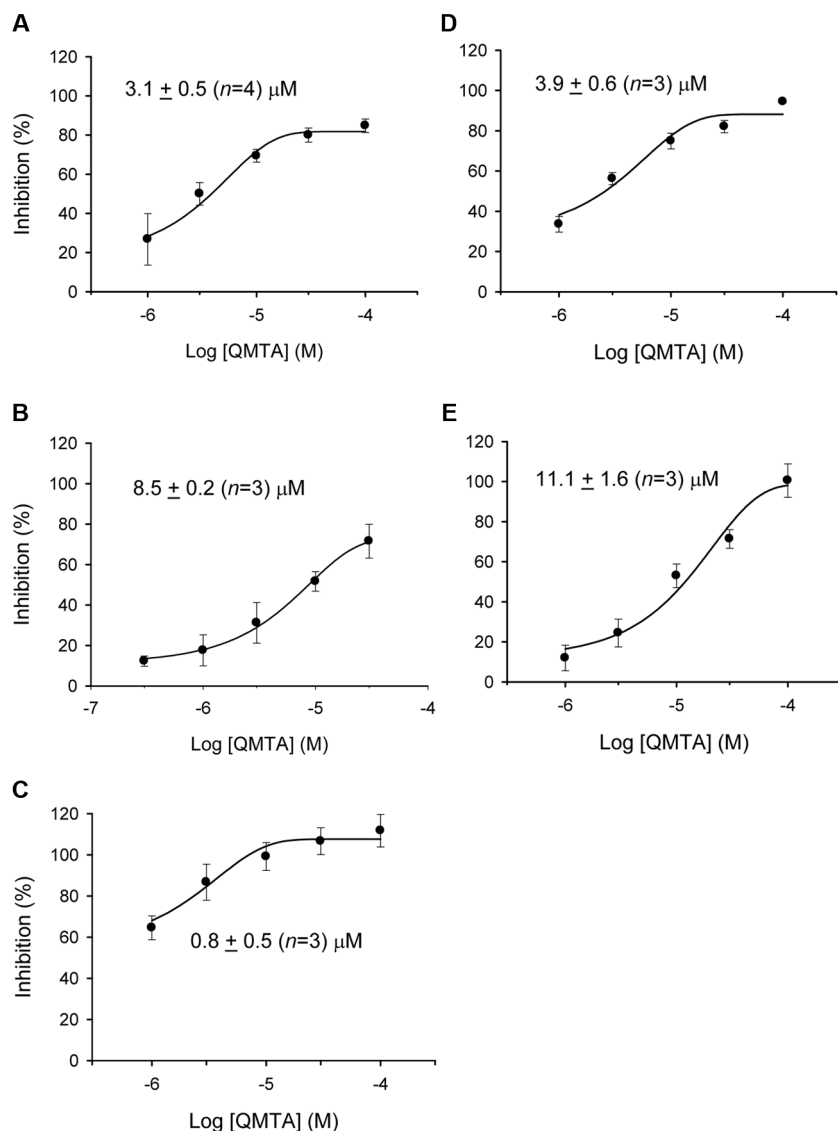


Fig. 4 Log concentration-inhibition curves of 3-O-methylquercetin 5,7,3',4'-O-tetraacetate (QMTA) on phosphodiesterase (PDE)1 (A), PDE2 (B), PDE3 (C), PDE4 (D), and PDE5 (E). Their IC_{50} values are shown in each respective graph. Each point represents the mean $\pm$ SEM ($n = 3$ or 4).

to the Lineweaver-Burk analysis, QMTA (1–10 μ M) and milrinone (0.3–3 μ M), a selective PDE3 inhibitor, competitively inhibited PDE3 activity (Fig. 5) because the $1/V_{max}$ values were not significantly affected by various concentrations of QMTA (A) or milrinone (B). Their K_i values were calculated to be 0.9 ± 0.3 ($n = 5$) and 2.5 ± 0.4 ($n = 7$) μ M, respectively (Fig. 5 insert). Similarly, QMTA (1–10 μ M) and rolipram (1–10 μ M), a selective PDE4 inhibitor, competitively inhibited PDE4 activity (Fig. 6). Their K_i values were calculated to be 3.9 ± 0.5 ($n = 5$) and 4.4 ± 1.8 ($n = 5$) μ M, respectively (Fig. 6 insert). The K_i value of QMTA for PDE3 was significantly less than that for PDE4, suggesting that QMTA has higher affinity for PDE3 than for PDE4.

Discussion

Allergic asthma is a chronic respiratory disease characterized by AHR, mucus hypersecretion, bronchial inflammation, and elevated serum immunoglobulin-E (IgE). T helper type 2 (Th2) cells, together with other inflammatory cells such as eosinophils, B cells, and mast cells have been proposed to play critical roles in the in-

itiation, development, and chronicity of this disease [22]. One hypothesis emphasizes an imbalance in the helper lymphocyte population favoring expression of Th2 over Th1 cells. Cytokines released from Th2 lymphocytes are IL-4, 5, 6, 9, and 13, and TNF- α , and those from Th1 lymphocytes are IL-2, 4, and 12, and IFN- γ [23]. In the present results, QMTA suppressed IL-2, 4, and 5, IFN- γ , and TNF- α , suggesting that QMTA selectively suppresses neither Th1 nor Th2 lymphocytes. QMTA also suppressed all types of inflammatory cells examined, including total inflammatory cells, macrophages, lymphocytes, neutrophils, and eosinophils from the BALF of sensitized and challenged mice. Even the eosinophils were abolished by QMTA at the various doses used.

Inflammatory mediators released during allergic airway inflammation play a role in the development of AHR. IL-4 and -13 have been shown to induce AHR in mouse asthma models [24]. IL-4 has three primary effects. First, IL-4 promotes B cell differentiation to plasma cells that secrete antigen-specific IgE antibodies. Second, IL-4 promotes mast cell proliferation. Third, increased IL-4 upregulates endothelial cellular expression of adhesion molecules for eosinophils [25]. IL-5 mobilizes and activates eosino-

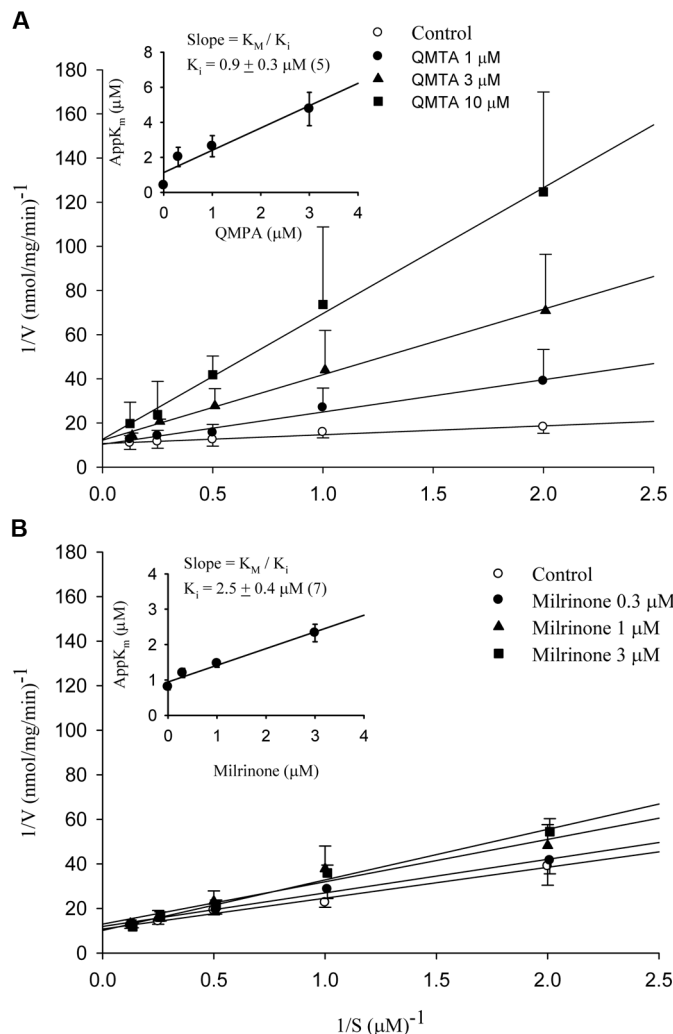


Fig. 5 Inhibition of phosphodiesterase (PDE)3-induced cAMP hydrolysis by 3-O-methylquercetin 5,7,3',4'-O-tetraacetate (QMTA) (**A**) and milrinone (**B**). The activities of PDE3 in the presence of various concentrations of QMTA or milrinone, and the substrate (cAMP) were plotted according to the Lineweaver-Burk analysis. K_i was determined from the equation of the apparent K_m as a function of the inhibitor concentration (insert). Each value represents the mean $\pm$ SEM ($n = 5-7$).

phils, leading to the release of a major basic protein, cysteinyl-leukotrienes, and eosinophil peroxidase both of which contribute to tissue damage and AHR [26]. The calcium channels in mast cell membranes have been proposed to differ from those in cardiovascular and other smooth muscle tissues [27], which are sensitive to nifedipine. In the present *in vitro* results, QMTA concentration-dependently inhibited the cumulative OVA-induced contractions of isolated sensitized guinea pig trachealis, which were unaffected by $1 \mu\text{M}$ nifedipine, suggesting that QMTA inhibits degranulation and activation of mast cells [4]. Therefore, this inhibition by QMTA, an acetate of 3-MQ, may be related to the non-specific property of quercetin as a protein kinase inhibitor [28]. In the isolated sensitized guinea pig trachealis, however, QMTA as well as nifedipine concentration-dependently relaxed the baseline, suggesting that QMTA has a bronchodilator effect.

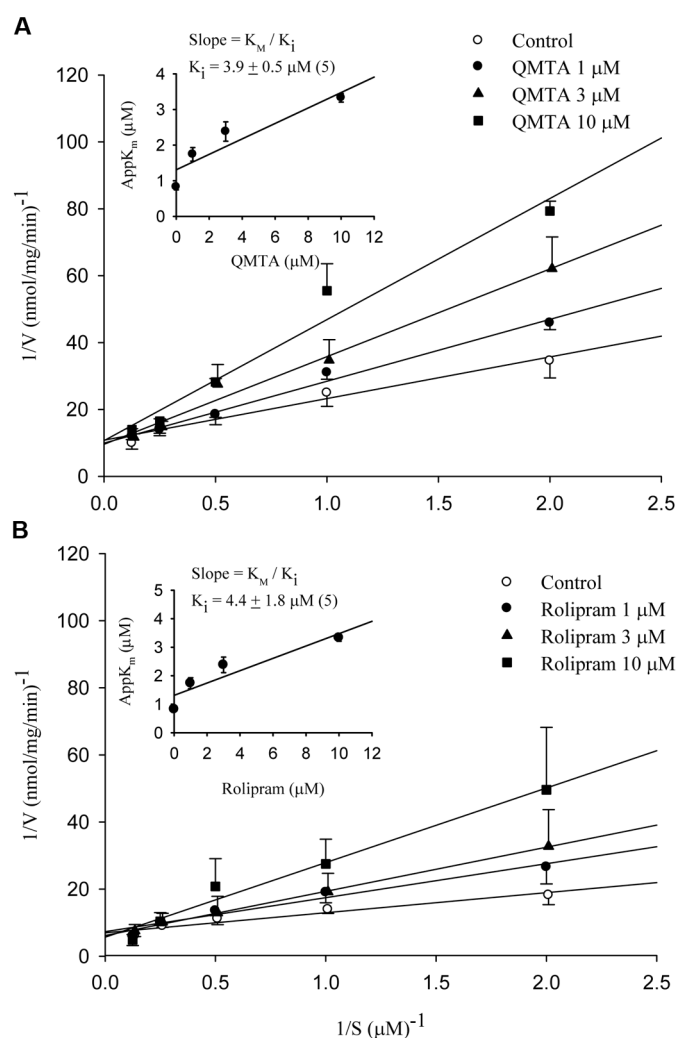


Fig. 6 Inhibition of phosphodiesterase (PDE)4-induced cAMP hydrolysis by 3-O-methylquercetin 5,7,3',4'-O-tetraacetate (QMTA) (**A**) and rolipram (**B**). The activities of PDE4 in the presence of various concentrations of QMTA or rolipram, and the substrate (cAMP) were plotted according to the Lineweaver-Burk analysis. K_i was determined from the equation of the apparent K_m as a function of the inhibitor concentration (insert). Each value represents the mean $\pm$ SEM ($n = 5$).

Cyclic AMP, a 3',5'-cyclic nucleotide, has important regulatory roles in the process of inflammation. Selective PDE4 inhibitors specifically prevent the hydrolysis of cAMP, and therefore have broad anti-inflammatory effects such as inhibition of cell trafficking, and cytokine and chemokine release from inflammatory cells. The effectiveness of these PDE4 inhibitors may be limited by their clinical potency when using doses that have minimal effects on nausea and vomiting [29]. Development of inhibitors of other PDE classes, combined with PDE4 inhibition, may be another way forward. A dual PDE3/4 inhibitory compound may provide more bronchodilator effects in addition to the beneficial effects of PDE4 [30]. Although QMTA, as well as 3-MQ [6], had higher affinity for PDE3 than for PDE4, and had more selective inhibitory effects on PDE3 than on PDE4 in the present results, QMTA had more potency than 3-MQ and even at $1 \mu\text{mol/kg}$ (*i.p.*) significantly suppressed the increases in IL-2, TNF- α and eosinophils from the BALF of control mice. It is possible that the liposolubility of QMTA is greater than that of 3-MQ. Therefore QMTA

more easily penetrates cell membranes than 3-MQ, because 3-MQ has four hydroxyl groups at C-5, -7, -3', and -4' in the chemical structure. It is well known that the absorption of polyphenols, such as flavonoids, by the body is very poor, therefore the total intracellular amount of QMTA may have been greater than that of 3-MQ at the same dose after absorption, although QMTA may have been hydrolyzed by esterase to form 3-MQ. The target of the anti-inflammatory effects of both compounds *in vivo* was PDE3/4 and appeared different from that *in vitro*, especially in inhibiting the effect on the degranulation of mast cells, but appeared the same to that *in vitro* in relaxing baseline tension.

In conclusion, the above results strongly suggest that QMTA has greater potency and a higher potential than 3-MQ for use in the treatment of allergic asthma.

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References

- Lee ME, Markowitz J, Lee JO, Lee H. Crystal structure of phosphodiesterase 4D and inhibitor complex (1). *FEBS Lett* 2002; 530: 53–8.
- de Boer J, Philpott AJ, van Amsterdam RG, Shahid M, Zaagsma J, Nicholson CD. Human bronchial cyclic nucleotide phosphodiesterase isoenzymes: biochemical and pharmacological analysis using selective inhibitors. *Br J Pharmacol* 1992; 106: 1028–34.
- Silver PJ, Hamel LT, Perrone MH, Bentley RG, Bushover CR, Evans DB. Differential pharmacologic sensitivity of cyclic nucleotide phosphodiesterase isozymes isolated from cardiac muscle, arterial and airway smooth muscle. *Eur J Pharmacol* 1988; 150: 85–94.
- Ko WC, Chen MC, Wang SH, Lai YH, Chen JH, Lin CN. 3-O-Methylquercetin more selectively inhibits phosphodiesterase subtype 3. *Planta Med* 2003; 69: 310–5.
- Underwood DC, Kotzer CJ, Bochnowicz S, Osborn RR, Luttmann MA, Hay DW et al. Comparison of phosphodiesterase III, IV and dual III/IV inhibitors on bronchospasm and pulmonary eosinophil influx in guinea pigs. *J Pharmacol Exp Ther* 1994; 270: 250–9.
- Chiu NY, Chang KH. The illustrated medicinal plants of Taiwan. 5. Taipei: Southern Materials Center; 1998: 135–6.
- Jiang JS, Shih CM, Wang SH, Chen TT, Lin CN, Ko WC. Mechanisms of suppression of nitric oxide production by 3-O-methylquercetin in RAW 264.7 cells. *J Ethnopharmacol* 2006; 103: 281–7.
- Ko WC, Shih CM, Chen MC, Lai YH, Chen JH, Chen CM et al. Suppressive effects of 3-O-methylquercetin on ovalbumin-induced airway hyper-responsiveness. *Planta Med* 2004; 70: 1123–7.
- Ferte J, Kuhnel JM, Chapuis G, Rolland Y, Lewin G, Schwaller MA. Flavonoid-related modulators of multidrug resistance: synthesis, pharmacological activity, and structure-activity relationships. *J Med Chem* 1999; 42: 478–89.
- Lin CN, Lu CM, Lin HC, Ko FN, Teng CM. Novel antiplatelet naphthalene from *Rhamnus nakaharui*. *J Nat Prod* 1995; 58: 1934–40.
- Kanehiro A, Ikemura T, Makela MJ, Lahn M, Joetham A, Dakhama A et al. Inhibition of phosphodiesterase 4 attenuates airway hyperresponsiveness and airway inflammation in a model of secondary allergen challenge. *Am J Respir Crit Care Med* 2001; 163: 173–84.
- Hamelmann E, Schwarze J, Takeda K, Oshiba A, Larsen GL, Irvin CG et al. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am J Respir Crit Care Med* 1997; 156: 766–75.
- Winterrowd GE, Chin JE. Flow cytometric detection of antigen-specific cytokine responses in lung T cells in a murine model of pulmonary inflammation. *J Immunol Methods* 1999; 226: 105–18.
- Thompson WJ, Appleman MM. Multiple cyclic nucleotide phosphodiesterase activities from rat brain. *Biochemistry* 1971; 10: 311–6.
- Ahn HS, Crim W, Romano M, Sybertz E, Pitts B. Effects of selective inhibitors on cyclic nucleotide phosphodiesterases of rabbit aorta. *Biochem Pharmacol* 1989; 38: 3331–9.
- Podzuweit T, Nennstiel P, Muller A. Isozyme selective inhibition of cGMP-stimulated cyclic nucleotide phosphodiesterases by erythro-9-(2-hydroxy-3-nonyl) adenine. *Cell Signal* 1995; 7: 733–8.
- Harrison SA, Reifsnnyder DH, Gallis B, Cadd GG, Beavo JA. Isolation and characterization of bovine cardiac muscle cGMP-inhibited phosphodiesterase: a receptor for new cardiotonic drugs. *Mol Pharmacol* 1986; 29: 506–14.
- Reeves ML, Leigh BK, England PJ. The identification of a new cyclic nucleotide phosphodiesterase activity in human and guinea-pig cardiac ventricle. Implications for the mechanism of action of selective phosphodiesterase inhibitors. *Biochem J* 1987; 241: 535–41.
- Gillespie PG, Beavo JA. Inhibition and stimulation of photoreceptor phosphodiesterases by dipyrindamole and M&B 22,948. *Mol Pharmacol* 1989; 36: 773–81.
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72: 248–54.
- Tsien RW. Calcium channels in excitable cell membranes. *Annu Rev Physiol* 1983; 45: 341–58.
- Busse WW, Lemanske RF Jr. Asthma. *N Engl J Med* 2001; 344: 350–62.
- Revets H, Pynaert G, Grooten J, De Baetselier P. Lipoprotein I, a TLR2/4 ligand modulates Th2-driven allergic immune responses. *J Immunol* 2005; 174: 1097–103.
- Taube C, Duez C, Cui ZH, Takeda K, Rha YH, Park JW et al. The role of IL-13 in established allergic airway disease. *J Immunol* 2002; 169: 6482–9.
- Tucker J, Fanta CH. Integrative inflammation pharmacology: asthma. In: Golan DE, Tashjian Jr AH, Armstrong EJ, Galanter JM, Armstrong AW, Arnaout RA et al., editors. Principles of pharmacology-the pathophysiologic basis of drug therapy Philadelphia: Lippincott Williams & Wilkins; 2005: 695–705.
- Vargaftig BB, Singer M. Leukotrienes mediate murine bronchopulmonary hyperreactivity, inflammation, and part of mucosal metaplasia and tissue injury induced by recombinant murine interleukin-13. *Am J Respir Cell Mol Biol* 2003; 28: 410–9.
- Murphy AW, Platts-Mills TAE. Drug used in asthma and obstructive lung disease. In: Brody TM, Larner J, Minneman KP, editors. Human pharmacology – molecular to clinical. St. Louis: Mosby; 1998: 797–825.
- Groundwater PW, Solomons KR, Drewe JA, Munawar MA. Protein tyrosine kinase inhibitors. *Prog Med Chem* 1996; 33: 233–329.
- Giembycz MA. Phosphodiesterase 4 inhibitors and the treatment of asthma: Where are we now and where do we go from here? *Drugs* 2000; 59: 193–212.
- Fan CK. Phosphodiesterase inhibitors in airways disease. *Eur J Pharmacol* 2006; 533: 110–7.