

Anti-inflammatory activity of quercetin and isoquercitrin in experimental murine allergic asthma

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Received 7 July 2006; returned for revision 12 September 2006; returned for final revision 12 March 2007; accepted by A. Falus 2 May 2007

Abstract. *Objective:* Eosinophils and cytokines are implicated in the pathogenesis of allergic diseases. In the present study, we investigate the anti-inflammatory effect of quercetin and isoquercitrin in a murine model of asthma.

Methods: BALB/c mice were immunized (ovalbumin/aluminum hydroxide, s.c.), followed by two intranasal ovalbumin challenges. From day 18 to day 22 after the first immunization, the mice received daily gavages of isoquercitrin (15 mg/kg) or quercetin (10 mg/kg). Dexamethasone (1 mg/kg, s.c.) was administered as a positive control. Leucocytes were analyzed in bronchoalveolar lavage fluid (BALF), blood and pulmonary parenchyma at 24 h after the last ovalbumin challenge. Interleukin-5 (IL-5) was analyzed in BALF and lung homogenates.

Results: In animals receiving isoquercitrin or quercetin, eosinophil counts were lower in the BALF, blood and lung parenchyma. Neutrophil counts in blood and IL-5 levels in lung homogenate were lower only in isoquercitrin-treated mice. No alterations in mononuclear cell numbers were observed.

Conclusion: Quercetin and isoquercitrin are effective eosinophilic inflammation suppressors, suggesting a potential for treating allergies.

Key words: Quercetin – Isoquercitrin – Eosinophils – Murine model of asthma

Introduction

Allergic asthma is a complex inflammatory disorder characterized by airway hyperresponsiveness, eosinophilic inflammation, and hypersecretion of mucus by goblet cells. It is accompanied by high serum levels of immunoglobulin E (IgE), as well as by intrapulmonary production of interleukin

(IL)-4, IL-5, and IL-13 by allergen-specific T-helper 2 (Th2) cells [1, 2]. In addition to its role in the activation of mature eosinophils, IL-5 is the principal Th2 cytokine involved in terminal differentiation and proliferation of eosinophil precursors [3, 4]. Furthermore, IL-5 is essential for eosinophil migration from the bone marrow into the blood [5, 6].

Pharmacologic compounds capable of inhibiting eosinophil function or infiltration are desirable for the treatment of patients with atopy/allergic disorders, hypereosinophilic syndromes, and other eosinophil-related diseases. A number of novel classes of drugs that inhibit eosinophil activation are currently under development, suggesting that there are different therapeutic targets, most of which are suppressed by corticosteroids. Corticosteroids increase the transcription of genes coding for anti-inflammatory proteins, including lipocortin-1, IL-10, IL-1 receptor antagonist and neutral endopeptidase. However, this is unlikely to account for the broad spectrum of anti-inflammatory effects exerted by corticosteroids. The most striking corticosteroid effect is that of inhibiting the expression of multiple inflammatory genes (cytokines, enzymes, receptors and adhesion molecules), which has a strong influence on leukocyte recruitment [7].

Medicinal plants have long been used in folk medicine as alternative treatments for a wide range of diseases, including inflammatory processes of diverse origins, and have produced relief of symptoms comparable to that obtained by allopathic medicines [8, 9]. Several plant-derived secondary metabolites have been shown to interfere directly with molecular mechanisms, such as the mediation of inflammatory responses and production/activity of second messengers, as well as the expression of transcription factors and key pro-inflammatory molecules [10].

Polyphenolic flavonoids isolated from natural sources are among a wide variety of phytochemicals present in the human diet [11]. Previous reports have described several flavonoids that have potent anti-allergic effects in murine models of asthma [12–14]. Quercetin, a flavonoid found in

a wide variety of plants and usually linked to sugars such as glucose (quercetin-3-glucoside; isoquercitrin) or rutinose (rutin), presents anti-inflammatory and anti-allergic properties [15]. Quercetin induces significant gene expression and production of Th-1-derived interferon (IFN)- γ , as well as downregulating Th-2-derived IL-4 production by normal peripheral blood mononuclear cells [16]. In a guinea pig model of asthma, quercetin prevented allergen-induced and platelet-activating factor-induced bronchial obstruction, as well as bronchial hyperreactivity [17]. Isoquercitrin also demonstrated anti-asthmatic activity by inhibiting of carbachol- and leukotriene D₄-induced contraction in guinea pig airways [18].

In the present study, we evaluate the therapeutic anti-inflammatory effect of quercetin and isoquercitrin in a murine model of asthma.

Materials and methods

Materials

Isoquercitrin was purchased from the Sigma Chemical Co. (St. Louis, MO, USA), and quercetin was isolated from *Dimorphandra mollis* by Produtos Vegetais do Piauí S.A. (Parnaíba, Brazil).

Animals

Female BALB/c mice (4–6 weeks old and weighing 15–20 g) were purchased from the animal facilities of the Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, and maintained under standard laboratory conditions (21–24 °C at 40–60 % relative humidity on a 12-h light/dark cycle). All experiments were approved by and conducted in accordance with the guidelines of the Animal Care Committee from the Universidade de São Paulo (protocol n°. 04.1.659.53.5).

Antigen immunization, booster, and airway challenge

Mice were immunized on days 0 and 7 by subcutaneous injection of 0.4 mL of saline containing 4 μ g of ovalbumin and 1.6 mg of aluminum hydroxide, followed by two intranasal ovalbumin challenges on day 14 and 21 with 10 μ g of ovalbumin in 50 μ L saline delivered into the nostrils under light ether anesthesia with the aid of a micropipette. Non-immunized mice received two intranasal instillations of ovalbumin (as described above) and were used as control group. All determinations were made 24 h after the last ovalbumin challenge (22 days after the first immunization) [19, 20].

Treatment with quercetin and isoquercitrin

In order to determine the therapeutic effects of quercetin and isoquercitrin in a murine model of asthma, the following protocol was adopted. Mice immunized (on days 0 and 7) and challenged (on day 14 and 21) with ovalbumin as described above were randomly divided into 5 groups, designated Ova-only (untreated mice), Ova+vehicle (mice receiving oral propylene glycol:water, 1:1), Ova+dexamethasone (mice receiving subcutaneous injections of dexamethasone), Ova+isoquercitrin (mice receiving oral isoquercitrin) and Ova+quercetin (mice receiving oral quercetin). Equimolar amounts of quercetin (10 mg/kg) or isoquercitrin (15 mg/kg), or vehicle (propylene glycol:water, 1:1) were administered daily by gavage (0.3 mL) to each group of mice ($n = 6$) from days 18 to 22 after the first immunization. One group treated with subcutaneous injections of dexamethasone (1 mg/kg, 0.25 mL) was used as a positive control of anti-inflammatory activity.

Evaluation of leukocyte influx into bronchoalveolar spaces

Mice were euthanized with sodium pentobarbitone. The chest cavity of each animal was carefully opened, after which the trachea was exposed and catheterized. The catheter was secured, and phosphate-buffered saline (PBS) containing 0.5 % sodium citrate was infused in three aliquots (0.3, 0.3 and 0.4 mL) in a total volume of 1 mL. The bronchoalveolar lavage fluid (BALF) was recovered and placed on ice. Total cell counts were immediately performed in a Neubauer chamber. Differential counts were obtained using Rosenfeld-stained cytopsin preparations. Following centrifugation (405 $\times$ g for 5 min at 4 °C), BALF supernatants were collected and stored at –70 °C for subsequent cytokine determinations.

Lung homogenates

Mice lungs were removed, weighed and homogenized (Ultra-Turrax T8; IKA-Werke, Staufen, Germany) in 1.0 mL PBS, centrifuged (405 $\times$ g for 5 min at 4 °C). The supernatants were collected and stored at –70 °C for subsequent cytokine determinations. Cytokine levels were determined per mg of tissue.

Determination of cytokines

Commercially available enzyme-linked immunosorbent assay antibodies were used to measure IL-5 and IFN- γ (BD Pharmingen, San Diego, CA, USA) in BALF and lung homogenates according to manufacturer instructions (BD Pharmingen). Sensitivities were >10 pg/mL.

Lung histology

Lungs were removed, immersed in 10 % phosphate-buffered formalin and embedded in paraffin. Tissue sections 5- μ m thick were stained with hematoxylin and eosin for light microscopy examination.

Statistical analysis

Data are presented as mean $\pm$ SEM. Multiple comparisons were performed through analysis of variance using Tukey's post-test. Probability (p) values of <0.05 were considered statistically significant.

Results

Effect of quercetin and isoquercitrin on blood, BALF and pulmonary parenchyma cell counts

Figure 1A shows the results of the differential blood cell counts, revealing that the numbers of eosinophils in the blood of animals that were immunized and then challenged with ovalbumin were 10-fold greater than those seen in the controls. No differences in neutrophil and mononuclear cell numbers were observed (Fig. 1B, C). In addition, there were no significant differences between the Ova+vehicle group mice and the Ova-only group mice in terms of the blood cell counts (Fig. 1A–C). Eosinophil numbers in the blood of mice in the Ova+isoquercitrin and Ova+quercetin groups were, respectively, 67 % and 64 % lower than those observed in the Ova-only group mice (Fig. 1A). Neutrophil recruitment was also 70 % lower in the Ova+isoquercitrin group (Fig. 1B). In the Ova+isoquercitrin and Ova+quercetin groups, no alterations in mononuclear cell numbers were observed (Fig. 1C). Furthermore, eosinophil and mononuclear cell counts were

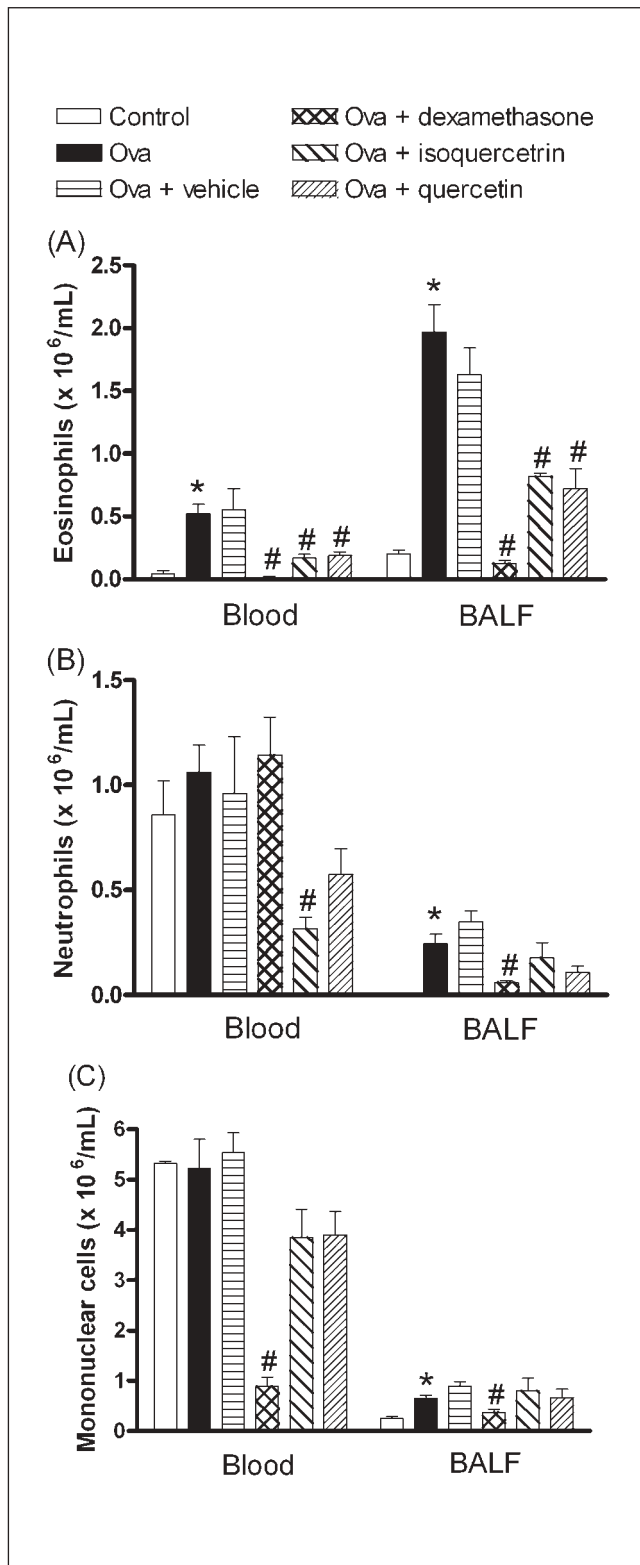


Fig. 1. Eosinophil numbers (A), neutrophil numbers (B) and mononuclear cell numbers (C) in the blood and BALF of animals treated with vehicle (propylene glycol:water, 1:1), dexamethasone (1mg/kg), isoquercitrin (15mg/kg) or quercetin (10mg/kg). Animals were treated daily from the 18th to the 22nd day after first immunization. Cell counts were performed 24h after the second ovalbumin challenge. Values are presented as means $\pm$ SEM (n = 6 per group). *p < 0.05 vs. controls; #p < 0.05 vs. Ova-only

significantly lower in the Ova+dexamethasone group than in the Ova-only group (Fig. 1A–C).

Figure 1 also demonstrates the result of cell infiltration into the BALF and the results obtained after ovalbumin-challenge are in agreement with those of previous studies [19, 20]. In the BALF of Ova-only group mice, there were significantly greater numbers of eosinophils, neutrophils and mononuclear cells than in that of the controls (Fig. 1A–C). No significant differences were observed between the Ova+vehicle group and the Ova-only group in terms of cell recruitment (Fig. 1A–C). Eosinophil numbers in the BALF of mice in the Ova+isoquercitrin and Ova+quercetin groups were, respectively, 58 % and 63 % lower than those observed in the that of mice in the Ova-only group (Fig. 1A). In those same groups, no alterations in neutrophil or mononuclear cell numbers were observed (Fig. 1B, C). Eosinophil, neutrophil and mononuclear cell counts in the BALF were significantly lower in the Ova+dexamethasone group than in the Ova-only group (Fig. 1A–C).

Histological studies revealed that the Ova-only group mice developed peribronchovascular inflammation (with eosinophilic infiltrates) after ovalbumin challenge, exhibiting severe airway inflammation. Similar results were observed in Ova+vehicle group mice. However, there was less leukocyte infiltration into the lung tissue of mice in the Ova+isoquercitrin, Ova+quercetin and Ova+dexamethasone groups than into that of those in the Ova-only group, and, of the infiltrates observed, most were eosinophilic (Fig. 2).

IL-5 and IFN- γ levels in bronchoalveolar lavage fluid and lung homogenates

Levels of IL-5 and IFN- γ were determined in the BALF and lung homogenate supernatants of all groups. No significant differences were found among groups founding terms of IFN- γ levels, with the exception of a reduction in IFN- γ levels observed in the BALF of mice in the Ova+dexamethasone group (data not shown). All immunized, ovalbumin-challenged mice presented significantly higher levels of IL-5 in the BALF (data not shown) and lung homogenates (Fig. 3) than did the control mice. The levels of IL-5 in the BALF observed in the Ova+isoquercitrin and Ova+quercetin groups were comparable to those seen in the Ova-only group (data not shown). In addition, IL-5 levels in the BALF and lung homogenates of Ova+vehicle group mice presented no significant differences in comparison with those seen in the Ova-only group mice. However IL-5 levels in the lung homogenates of Ova+isoquercitrin mice were 43 % lower than those observed in the lung homogenates of Ova-only group mice (Fig. 3). No significant alterations in IL-5 levels were observed in the Ova+quercetin group. In comparison with the Ova-only group mice, those in the Ova+dexamethasone group presented significantly lower levels of IL-5 in the BALF (data not shown) and lung homogenates (Fig. 3).

Discussion

Eosinophilia is a hallmark of allergic and parasitic diseases. Eosinophils are an important source of a various regulatory cytokines (tumor necrosis factor- α , granulocyte-macrophage

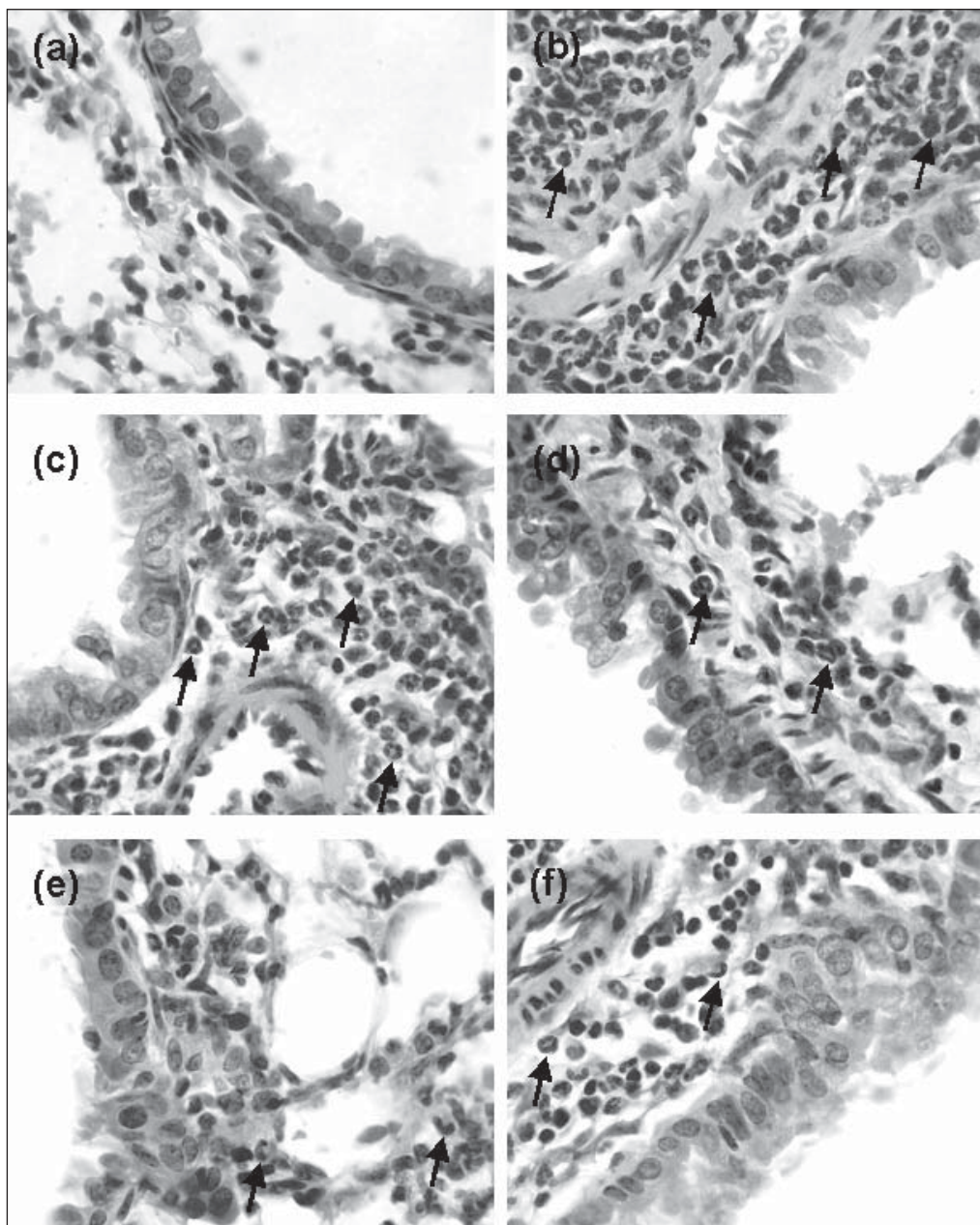


Fig. 2. Lung histopathology. Photomicrograph showing the normal lung parenchyma of control mice (A) without inflammatory cells; of ovalbumin-sensitized, ovalbumin-challenged, untreated mice (B) and of ovalbumin-sensitized, ovalbumin-challenged mice treated with vehicle (C), the last presenting inflammatory cell infiltration into the peribronchial and perivascular areas. Treatment with dexamethasone (D), isoquercitrin (E) or quercetin (F) provoked no significant inflammatory cell infiltration into the bronchovascular area. Arrows indicate eosinophils in the lung. Magnification: $\times 1000$

colony-stimulating factor, IL-4, IL-13 and IL-5) [21–24] and chemokines (CXCL8, CCL5 and CCL11) [25–27], which have pro-inflammatory and immunoregulatory functions and properties. Eosinophils also produce cysteinyl leukotrienes [28, 29], which are the leading cause of the bronchoconstriction, mucus hypersecretion and increased vascular permeability seen in asthma sufferers [30, 31]. In addition, eosinophils release four principal cationic proteins (major basic protein, eosinophil-derived neurotoxin, eosinophil cationic protein and eosinophil peroxidase), all of which can damage lung tissue [32].

Glucocorticoids are widely used for the suppression of inflammation in chronic inflammatory diseases such as asthma, rheumatoid arthritis, inflammatory bowel disease and autoimmune diseases, all of which are associated with

increased expression of inflammatory genes [33–35]. Dexamethasone is routinely used to inhibit cell migration to the sites of inflammation, and this inhibition is accomplished, in part, by its potent effect on the synthesis of pro-inflammatory cytokines and chemokines, together with its capacity to reduce the degree of leukocyte responsiveness [36]. The anti-inflammatory action of glucocorticoids is thought to be a crucial component of their efficacy in treating allergic diseases, although this is tempered by their side-effects. Therefore, it would be desirable to create a compound that acts like a steroid but has fewer side effects [37]. The potential of natural products to inhibit eosinophilic inflammation was recently demonstrated, both in experimental animal models [38, 39] and in humans [40]. Many flavonoids have been found to exert potent anti-allergic and anti-inflammatory effects. Pre-

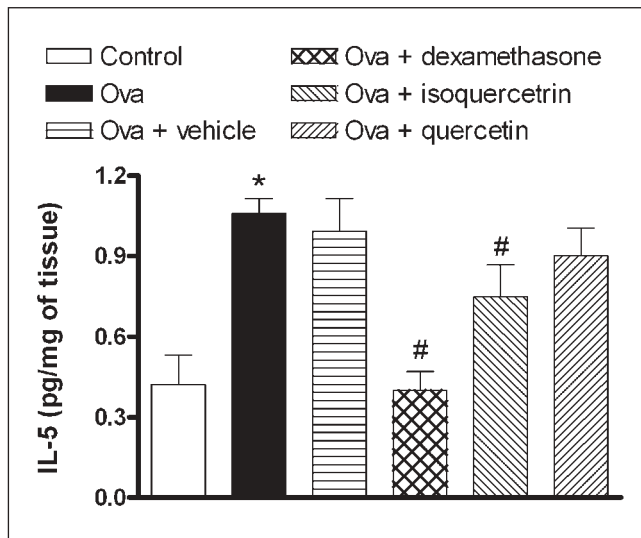


Fig. 3. IL-5 in lung homogenates of animals treated daily with vehicle (propylene glycol:water, 1:1), dexamethasone (1 mg/kg), isoquercitrin (15 mg/kg) or quercetin (10 mg/kg) from the 18th to the 22nd day after the first immunization. Experiments were performed 24 h after the second ovalbumin challenge. Values are presented as means $\pm$ SEM ($n = 6$ per group). * $p < 0.05$ vs. controls; # $p < 0.05$ vs. Ova-only

vious studies have shown that flavonoids alleviate bronchoconstriction and airway hyperreactivity in ovalbumin-sensitized mice [41]. In addition, flavonoids have been shown to inhibit the development of dermatitis and elevated IgE levels in a murine model of allergy [42]. Furthermore, flavonoids suppress the production of Th2-type cytokines by activated human basophils [43]. Quercetin and isoquercitrin have been shown to inhibit the bronchial obstruction induced by different stimuli in models of allergic asthma [17, 18]. However, the anti-inflammatory activity of quercetin and isoquercitrin has never been studied in a murine model of asthma.

In the present study, we demonstrated that mice immunized and then challenged with ovalbumin exhibit lower numbers of eosinophils in the blood, BALF (Fig. 1A) and pulmonary parenchyma (Fig. 2) after being treated with isoquercitrin or quercetin in comparison with those receiving no treatment. In all experiments, dexamethasone was more effective in indiscriminately reducing the numbers of leukocytes. Since IL-5 is the principal Th2 cytokine involved in the terminal differentiation and proliferation of eosinophil precursors, as well as in the activation of mature eosinophils [6, 7] and in the promotion of eosinophil migration from the bone marrow into blood [4, 5], we analyzed IL-5 levels in order to study the effect of quercetin and isoquercitrin on eosinophil numbers. Curiously, only isoquercitrin reduced IL-5 levels in lung homogenates (Fig. 3), and neither quercetin nor isoquercitrin reduced IL-5 levels in the BALF (data not shown), suggesting that these compounds may inhibit others eosinophilic mediators, such as eotaxin [44].

The reduction in eosinophil recruitment by quercetin and isoquercitrin might be attributable to the inhibition of other inflammatory mediators, since quercetin downregulates the NF- κ B pathway [45]. Inhibition of the NF- κ B pathway is as-

sociated with suppression of the expression of genes encoding chemokines (IL-8, RANTES and eotaxin) and adhesion molecules (intercellular adhesion molecule, vascular cell adhesion molecule and E-selectin), all of which play critical roles in controlling eosinophil recruitment [46, 47]. However, neutrophils are a potential source of inflammatory mediators such as leukotriene B₄ (LTB₄) and platelet-activating factor (PAF) [48] which can also increase eosinophil recruitment [49]. It has been demonstrated that activation and recruitment of neutrophils can be blocked efficiently by compounds with scavenger/antioxidant properties [50, 51]. The reduction in eosinophil recruitment might also be associated with the inhibitory effect that quercetin and isoquercitrin (via reactive oxygen species-dependent molecular pathways) have on neutrophil activation [52, 53].

A variety of pharmacological agents, such as PAF receptor antagonists and 5-lipoxygenase inhibitors, have been shown to have inhibitory effects on *in vivo* eosinophil accumulation [37]. In addition, it has been demonstrated that quercetin and isoquercitrin, respectively, reduce PAF-induced and leukotriene-induced bronchoconstriction in a murine model of asthma [17, 18]. Therefore, it is possible that quercetin and isoquercitrin inhibit PAF and leukotriene production in cells.

The results of the present study show that quercetin and isoquercitrin both attenuate inflammation in a murine model of asthma. The effects described herein, as well as those observed by others investigators, together with the broad spectrum of the biological effects of these substances, strongly suggest that quercetin and isoquercitrin have therapeutic potential for the treatment of asthma and other allergic diseases. Therefore, the study of these two substances could lead to the development of an effective anti-asthma therapy or of a means of identifying novel anti-asthma targets.

Acknowledgments. This study received financial support in the form of grants from the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq, National Council for Scientific and Technological Development; Grant no. 472601/2004-0), the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES, Coordination of the Advancement of Higher Education) and the *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP, Foundation for the Support of Research in the state of São Paulo). We are grateful to technician Ana Maria da Rocha from the Departamento de Patologia (for help in the formulation of histologic preparations), and Dr. Carlos A. Lorgi from the Departamento de Análises Clínicas, Toxicológicas e Bromatológicas.

References

- [1] Anderson GP, Coyle AJ. Th2 and 'Th2-like' cells in allergy and asthma: pharmacological perspectives. *Trends Pharmacol Sci* 1994; 15: 324–32.
- [2] Wills-Karp M. Immunologic basis of antigen-induced airway hyperresponsiveness. *Annu Rev Immunol* 1999; 17: 255–81.
- [3] Yamaguchi Y, Suda T, Suda J, Eguchi M, Muira Y, Harada N et al. Purified interleukin-5 (IL-5) supports the terminal differentiation and proliferation of murine eosinophilic precursors. *J Exp Med* 1988; 167: 43–56.
- [4] Sanderson CJ. Interleukin-5, eosinophils, and disease. *Blood* 1992; 79: 3191–9.
- [5] Faccioli LH, Mokwa VF, Silva CL, Rocha GM, Araujo JI, Nahori MA et al. IL-5 drives eosinophils from bone marrow to blood and

- tissues in a guinea-pig model of visceral larva migrans syndrome. *Mediators Inflamm* 1996; 5: 24–31.
- [6] Rogerio AP, Sa-Nunes A, Albuquerque DA, Anibal FF, Medeiros AI, Machado ER et al. *Lafoensia pacari* extract inhibits IL-5 production in toxocariasis. *Parasite Immunol* 2003; 25: 393–400.
 - [7] Barnes PJ. Corticosteroids: the drugs to beat. *Eur J Pharmacol* 2006; 533: 2–14.
 - [8] Verpoorte R. Exploration of nature's chemodiversity: the role of secondary metabolites as leads in drug development. *Drug Discov Today* 1999; 3: 232–8.
 - [9] Clardy J, Walsh C. Lessons from natural molecules. *Nature* 2004; 432: 729–37.
 - [10] Calixto JB, Campos MM, Otuki MF, Santos ARS. Anti-inflammatory compounds of plant origin. Part II. modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. *Planta Med* 2003; 69: 973–83.
 - [11] Middleton E, Chithan K, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol Rev* 2000; 52: 673–751.
 - [12] Das M, Ram A, Ghosh B. Luteolin alleviates bronchoconstriction and airway hyperreactivity in ovalbumin sensitized mice. *Inflamm Res* 2003; 52: 101–6.
 - [13] Ko WC, Shih CM, Chen MC, Lai YH, Chen JH, Chen CM et al. Suppressive effects of 3-O-methylquercetin on ovalbumin-induced airway hyperresponsiveness. *Planta Med* 2004; 70: 1123–7.
 - [14] Wu YQ, Zhou CH, Tao J, Li SN. Antagonistic effects of nobletin, a polymethoxyflavonoid, on eosinophilic airway inflammation of asthmatic rats and relevant mechanisms. *Life Sci* 2006; 78: 2689–96.
 - [15] Gabor M. Anti-inflammatory and antiallergic properties of flavonoids. *Prog Clin Biol Res* 1986; 213: 471–80.
 - [16] Nair MP, Kandaswami C, Mahajan S, Chadha KC, Chawda R, Nair H et al. The flavonoid, quercetin, differentially regulates Th-1 (IFN- γ) and Th-2 (IL-4) cytokine gene expression by normal peripheral blood mononuclear cells. *Biochim Biophys Acta* 2002; 1593: 29–36.
 - [17] Dorsch W, Bittinger M, Kaas A, Muller A, Kreher B, Wagner H. Antiasthmatic effects of *Galphimia glauca*, gallic acid, and related compounds prevent allergen- and platelet-activating factor-induced bronchial obstruction as well as bronchial hyperreactivity in guinea pigs. *Int Arch Allergy Immunol* 1992; 97: 1–7.
 - [18] Fernandez J, Reyes R, Ponce H, Oropeza M, Vancalsteren MR, Jankowski C et al. Isoquercitrin from *Argemone platyceras* inhibits carbachol and leukotriene D₄-induced contraction in guinea-pig airways. *Eur J Pharmacol* 2005; 522: 108–15.
 - [19] Russo M, Nahori MA, Lefort J, Gomes E, De Castro Keller A, Rodriguez D et al. Suppression of asthma-like responses in different mouse strains by oral tolerance. *Am J Respir Cell Mol Biol* 2001; 24: 518–26.
 - [20] Rodriguez D, Keller AC, Faquim-Mauro EL, de Macedo MS, Cunha FQ, Lefort J et al. Bacterial lipopolysaccharide signaling through Toll-like receptor 4 suppresses asthma-like responses via nitric oxide synthase 2 activity. *J Immunol* 2003; 171: 1001–8.
 - [21] Dubucquoi S, Janin A, Desreumaux P, Rigot JM, Copin MC, Francois M et al. Evidence for eosinophil activation in eosinophilic cystitis. *Eur Urol* 1994; 25: 254–8.
 - [22] Levi-Schaffer F, Lacy P, Severs NJ, Newman TM, North J, Gomperts B et al. Association of granulocyte-macrophage colony-stimulating factor with the crystalloid granules of human eosinophils. *Blood* 1995; 85: 2579–86.
 - [23] Moller GM, de Jong TA, Van der kwast TH, Overbeek SE, Wierenga-Wolf AF, Thepen T et al. Immunolocalization of interleukin-4 in eosinophils in the bronchial mucosa of atopic asthmatics. *Am J Respir Cell Mol Biol* 1996; 14: 439–43.
 - [24] Woerly G, Lacy P, Younes AB, Roger N, Loiseau S, Moqbel R et al. Human eosinophils express and release IL-13 following CD28-dependent activation. *J Leukoc Biol* 2002; 72: 769–79.
 - [25] Braun RK, Franchini M, Erard F, Rihs S, De Vries IJ, Blaser K et al. Human peripheral blood eosinophils produce and release interleukin-8 on stimulation with calcium ionophore. *Eur J Immunol* 1993; 23: 956–60.
 - [26] Lim KG, Wan HC, Bozza PT, Resnick MB, Wong DT, Cruikshank WW et al. Human eosinophils elaborate the lymphocyte chemoattractants. IL-16 (lymphocyte chemoattractant factor) and RANTES. *J Immunol* 1996; 156: 2566–70.
 - [27] Nakajima T, Yamada H, Iikura M, Miyamasu M, Izumi S, Shida H et al. Intracellular localization and release of eotaxin from normal eosinophils. *FEBS Lett* 1998; 434: 226–30.
 - [28] Weller PF, Lee CW, Foster DW, Corey DM, Austen KF, Lewis RA. Generation and metabolism of 5-lipoxygenase pathway leukotrienes by human eosinophils: predominant production of leukotriene C₄. *Proc Natl Acad Sci USA* 1983; 80: 7626–30.
 - [29] Bandeira-Melo C, Weller PF. Eosinophils and cysteinyl leukotrienes. *Prostagl Leukot Essent Fatty Acids* 2003; 69: 135–43.
 - [30] Drazen JM. Leukotrienes as mediators of airway obstruction. *Am J Respir Crit Care Med* 1998; 158: S193–200.
 - [31] Holgate ST, Sampson AP. Antileukotriene therapy. Future directions. *Am J Respir Crit Care Med* 2000; 161: 147–53.
 - [32] Abu-Ghazaleh RI, Kita H, Gleich GJ. Eosinophil activation and function in health and disease. *Immunol Ser* 1992; 57: 137–67.
 - [33] Eum SY, Creminon C, Haile S, Lefort J, Vargaftig BB. Inhibition of airways inflammation by dexamethasone is followed by reduced bronchial hyperreactivity in BP2 mice. *Clin Exp Allergy* 1996; 26: 971–9.
 - [34] Frieri M. Corticosteroid effects on cytokines and chemokines. *Allergy Asthma Proc* 1999; 20: 147–59.
 - [35] Belvisi MG, Hele DJ. Soft steroids: a new approach to the treatment of inflammatory airways diseases. *Pulm Pharmacol Ther* 2003; 16: 321–5.
 - [36] van der Velden, VH. Glucocorticoids: mechanism of action and anti-inflammatory potential in asthma. *Mediators Inflamm* 1998; 7: 229–37.
 - [37] Teixeira M, Williams TJ, Hellewell PG. Mechanisms and pharmacological manipulation of eosinophil accumulation *in vivo*. *Trends Pharmacol Sci* 1995; 16: 418–23.
 - [38] Rogerio AP, Sa-Nunes A, Albuquerque DA, Anibal FF, Medeiros AI, Machado ER et al. *Lafoensia pacari* extract inhibits IL-5 production in toxocariasis. *Parasite Immunol* 2003; 25: 393–400.
 - [39] Sy LB, Wu YL, Chiang BL, Wang YH, Wu WM. Propolis extracts exhibit an immunoregulatory activity in an OVA-sensitized airway inflammatory animal model. *Int Immunopharmacol* 2006; 6: 1053–60.
 - [40] Gupta I, Gupta V, Parihar A, Gupta S, Ludtke R, Safayhi H et al. Effects of *Boswellia serrata* gum resin in patients with bronchial asthma: results of a double-blind, placebo-controlled, 6-week clinical study. *Eur J Med Res* 1998; 3: 511–4.
 - [41] Das M, Ram A, Ghosh B. Luteolin alleviates bronchoconstriction and airway hyperreactivity in ovalbumin sensitized mice. *Inflamm Res* 2003; 52: 101–6.
 - [42] Kotani M, Matsumoto M, Fujita A, Higa S, Wang W, Suemura M et al. Persimmon leaf extract and astragalin inhibit development of dermatitis and IgE elevation in NC/Nga mice. *J Allergy Clin Immunol* 2000; 106: 159–66.
 - [43] Hirano T, Higa S, Arimitsu J, Naka T, Shima Y, Ohshima S et al. Flavonoids such as luteolin, fisetin and apigenin are inhibitors of interleukin-4 and interleukin-13 production by activated human basophils. *Int Arch Allergy Immunol* 2004; 134: 135–40.
 - [44] Conroy DM, Williams TJ. Eotaxin and the attraction of eosinophils to the asthmatic lung. *Respir Res* 2001; 2: 150–6.
 - [45] Nam, NH. Naturally occurring NF- κ B inhibitors. *Mini Rev Med Chem* 2006; 6: 945–51.
 - [46] Yamamoto H, Nagata M, Sakamoto Y. CC chemokines and transmigration of eosinophils in the presence of vascular cell adhesion molecule 1. *Ann Allergy Asthma Immunol* 2005; 94: 292–300.
 - [47] Jia GQ, Gonzalo JA, Hidalgo A, Wagner D, Cybulsky M, Gutierrez-Jia GQ et al. Selective eosinophil transendothelial migration triggered by eotaxin via modulation of Mac-1/ICAM-1 and VLA-4/VCAM-1 interactions. *Int Immunol* 1999; 11: 1–10.
 - [48] Hampton MB, Kettle AJ, Winterbourn CC. Inside the neutrophil phagosome: oxidants, myeloperoxidase, and bacterial killing. *Blood* 1998; 92: 3007–17.
 - [49] Oliveira SH, Costa CH, Ferreira SH, Cunha FQ. Sephadex induces eosinophil migration to the rat and mouse peritoneal cavity: in-

- volvement of mast cells, LTB₄, TNF- α , IL-8 and PAF. *Inflamm Res* 2002; 51: 144–53.
- [50] Woo CH, Yoo MH, You HJ, Cho SH, Mun YC, Seong CM et al. Transepithelial migration of neutrophils in response to leukotriene B₄ is mediated by a reactive oxygen species-extracellular signal-regulated kinase-linked cascade. *J Immunol* 2003; 170: 6273–9.
- [51] Scott DA, Poston RN, Wilson RF, Coward PY, Palmer RM. The influence of vitamin C on systemic markers of endothelial and inflammatory cell activation in smokers and non-smokers. *Inflamm Res* 2005; 54: 138–44.
- [52] Jackson JK, Higo T, Hunter WL, Burt HM. The antioxidants curcumin and quercetin inhibit inflammatory processes associated with arthritis. *Inflamm Res* 2006; 55: 168–75.
- [53] Bosse WW, Kopp DE, Middleton EH. Flavenoid modulation of human neutrophil function. *J Allergy Clin Immunol* 1984; 23: 801–6.

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