Effects of pentagamavunon-0 on histamine-mediated hyperresponsive airway in asthmatic models : *in-vitro in-vivo*

Pengaruh pentagamavunon-0 terhadap sistem pernafasan hiperresponsif yang diperantarai histamin pada model asma *in-vitro in-vivo*

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Abstract

Asthma is a chronic inflammatory airway disease involving reversible airway constriction and airway hyperresponsiveness (AHR) to allergens, airway edema, and increased mucus secretion tumor cells. To date, exploration of antiasthmatic drug is still being studied both from natural products or sinthetic processes. One of the sinthetic compound is pentagamavunon-0 (PGV-0) which possesses anti-inflammatory and inhibitory effects on histamine release from rat mast cells. The aim of the study is to look at the effects of PGV-0 on histamine-mediated hyperresponsive airway in asthmatic models (*in vitro* and *in vivo* studies). In vitro study was conducted using isolated organ technique with isotonic transducer. The results have shown that PGV-0 could not inhibit the contraction of isolated guinea pig trachea induced by histamine. PGV-0 did not change the pD_2 and Emax values of histamine on trachea smooth muscle. The finding indicates that PGV-0 does not have affinity and intrinsic activity on H-1 histaminergic receptor in trachea smooth muscle. In vivo study, we sensitized the rats with ovalbumin (OVA) to develop the airway hyperreactivity to histamine. Histamine level in bronchoalveolar lavage fluid (BALF) and airway tissue were determined using HPLC-fluorometry. Multiple exposures of ovalbumin significantly histamine level in BALF by 74.51±5.33 pmol/mL or 6-times higher than this of control saline group. Oral administration of PGV-0 (40 mg/kg BW) significantly decreased the histamine accumulation in BALF to 30 % of the value of control group in asthmatic rats. Besides, PGV-0 significantly prevented the histamine decrease in asthmatic rats to 37.8 % trachea, and 34.2 % in bronchus. However, PGV-0 did not succeed to prevent the histamine decrease in the lung of asthmatic rats. The result of the study may provide useful information for further discovering pharmacological synthetic compound for treatment of allergic inflammatory asthma.
Key words : asthma, curcumin, pentamavunon-0, histamine, airway hyperresponsiveness

Abstrak

Asma merupakan penyakit sistem pernafasan bersifat kronik melibatkan konstriksi terbalikkan dan hiperresponsivitas pada sistem pernafasan. Pengembangan obat anti asma terus dikerjakan baik dari tanaman obat ataupun upaya sintesis. Salah satu kandidat senyawa yang akan dikembangkan adalah pentagamavunon-0 (PGV-0). Penelitian ini bertujuan untuk mempelajari pengaruh PGV-0 terhadap kontraksi otot polos trakea diinduksi histamin, dan terhadap konsentrasi histamine pada sistem pernafasan yang hiperresponsif akibat paparan alergen.

Penelitian in vitro dikerjakan dengan menggunakan teknik organ terisolasi menggunakan transduser isotonik. Pada penelitian ini, PGV-0 tidak mempengaruhi kontraksi otot polos trakea marmut yang diinduksi oleh agonis histamin. PGV-0 tidak mengubah harga pD_2 dan Emaks histamin pada otot polos trakea. Ini mengindikasikan bahwa PGV-0 tidak mempunyai afinitas maupun aktivitas intrinsik pada reseptor histaminik H1 yang terdapat pada otot polos trakea. Pada penelitian in vivo digunakan ovalbumin untuk merangsang hipersensitivitas sistem pernafasan. Konsentrasi histamin dalam *bronchoalveolar lavage fluid* (BALF) dan jaringan sistem pernafasan ditetapkan menggunakan HPLC-fluorometry. Paparan ovalbumin pada tikus dapat menstimulasi kenaikan konsentrasi histamin dalam BALF sebesar 74,51± 5,33 pmol/mL (6 kali dari nilai kelompok kontrol). Pemberian PGV-0 dosis 40 mg/kg BB mampu menurunkan kenaikan konsentrasi histamin yang distimulasi ovalbumin hingga 30 %. Di samping itu, pemberian PGV-0 juga mencegah penurunan histamin dalam jaringan sistem pernafasan terutama trakea dan bronkus masing-masingh sebesar 37,8 % dan 34,2 %. Namun, PGV-0 tidak mampu mencegah penurunan histamin dalam paru-paru. Ini berarti PGV-0 mencegah degranulasi sel mast di jaringan tersebut. Penelitian ini diharapkan dapat menyumbangkan ilmu pengetahuan bagi pengembangan obat anti asma.

Kata kunci : asma, kurkumin, pentagamavunon-0, histamin, hiperresponsivitas sistem pernafasan

Introduction

Pentagamavunon-0 (PGV-0), 2,5-bis(4'-hydroxy-3'-methoxy benzylidene) cyclopentanone (Fig. 1) has been synthesized for a number of purposes by Kodak in 1961 for developing film-forming photosensitive polymers (Kodak, 1961). This compound was also reported to have various pharmacological properties including antibacterial and antifungal effects as well as anti-inflammatory effect through inhibition of prostaglandin biosynthesis in cyclooxigenase pathway. In addition to these effects, PGV-0 also inhibited lipid peroxidation and functioned as a free hydroxy radical scavenger. The anti-oxidative, and anti-cyclooxygenase activities of this compound at dose of 20 mg/kg, orally, was 2- and 7-times stronger than those imposed by curcumin while its anti-inflammatory activity was reported to be 5-times higher (Sardjiman, 2000).

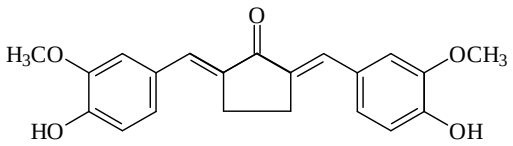

Figure 1. The chemical structure of Pentagamavunon-0.

Asthma pathogenesis is associated with inflammation of the airway wall, and also with airway hyperresponsiveness (AHR) to a variety of physical and pharmacological stimuli. Many cells and cellular elements play a role in asthma pathogenesis are mast cells, eosinophils, T lymphocytes, macrophages, neutrophils, and epithelial cells. Allergic asthma response begins when a processed allergen in presented to naive Th-precursor cells favors the selective expansion of Th2-polarized memory cells. Then, re-exposure of allergen can trigger early-phase airway response by cross-linkage of allergen into IgE antibody molecules on FcεRI receptors, and in turn can stimulate the release of histamine, cytokines and chemokines. This process is followed by late-phase airway responses after several hours. Eosinophils and Th2 produce inflammatory mediators such as IL-3, IL-5, GM-CSF. Mast cells, basophils, macrophages, neutrophils and platelets may also contribute to generate the release of inflammatory mediators (Holt *et al.,* 1999; Brunton *et al.,* 2008).

In our study, we investigated the effects of PGV-0 on isolated trachea smooth muscle of guinea pig stimulated by histamine, and the histamine level of allergen-induced airway hyperresponsiveness in sensitized rats.

We sensitized the rats with ovalbumin to develop the airway hyperreactivity to histamine. Histamine level in bronchoalveolar lavage fluid (BALF) and airway tissue were determined using HPLC-fluorometry.

Methodology

PGV-0 was kindly supplied by Dr. Supardjan A. Margono (Faculty of Pharmacy, Gadjah Mada University). Ovalbumin and histamine were purchased from Sigma-Aldrich (St. Louis USA), o-phthalaldehyde was from Wako Pure Chemical Industries (Osaka, Japan). Wistar rats weighing 180-230 g, and guinea pigs weighing 300-450 g were used. They were housed at a constant temperature (22±2 °C) with a constant relative humidity (55±10 %) on an automatically controlled 12:12 h light-dark cycle (light on at 7:00 a.m.) and has free access to food and water.

Contraction of Isolated trachea smooth muscle

The experiment was according to Sayah *et al.* (1998). Briefly, guinea-pigs were sacrificed by a blow on the head and were exsanguinated from carotid arteries. The trachea was rapidly removed and after being freed from connecting tissues was cut into transverse rings. The ventral cartilage of the rings was cut and strip-like preparations of tracheal smooth muscle were mounted in a 37 °C organ bath containing 200 mL Krebs Buffer solution, bubbled with carbogen gas (95 % 0_2 and 5 % CO_2). To induce contraction of tracheal smooth muscle, histamin solutions ranging 2.10^{-2} to 2.10^{-7} M were used. Whereas, the trachea strip was pre-incubated for 15 min with PGV-0 solution prior to single concentrations of histamine. Isotonic contractions were recorded by a level transducer (tipe 368, HSE, W. Germany) connected to a recorder (Kipp and Zonen BBD 41, The Netherlands).

Ovalbumin Sensitization Protocol

Sensitization method basically was according to Liu *et al.* (2005). Briefly, rats were sensitized by subcutaneous injection of 1.0 mL of 10 mg/mL ovalbumin mixed with 5 % aluminium hydroxide suspension in physiologic saline. At the same time, an intraperitoneal injection of Bordetella pertussis antigen solution containing 1.0 x 10^9 organisms/mL was given as an adjuvant. A subcutaneous injection of 1.0 mL of 1.0 mg/mL ovalbumin suspended in 10% aluminium hydroxide and physiologic saline was infected after 7 days for a booster purpose. The animals were intranasally challenged with 1 % ovalbumin suspension in saline for 10 minutes on days 14, 15 and 16. Final step was provocation of airway hyperresponsiveness. The rats were placed in an unrestrained whole-body plethysmographic chamber (Buxco Electronics, Sharon, Connecticut USA), and inhalated with 5 % ovalbumin in saline for 10 minutes on day 17. The constriction of the airway was shown as enhanced pause (Penh), and monitored for 60 minutes after last inhalation. Penh is an indicator unit of bronchconstriction or airway hyperreactivity in unrestrained plethysmography (Lomask, 2006). PGV-0 (40 mg/kg body weight, 1 mL volume) was administered orally to rats daily during sensitization period to evaluate the prevention effect of the compound.

Release histamine determination in bronchoalveolar lavage fluid (BALF)

After challenge with ovalbumin, rats were anesthetized by nembutal and BALF was collected via tracheal cannulation using 8 mL of phosphate buffered saline solution. Collections of BALF were conducted at least 3 times. Supernatants obtained by centrifugation of BALF at 250 x g for 10 min. Then, histamine levels in the supernatants were determined by HPLC-fluorometry (Yamatodani *et al.* 1985). The histamine was post-labeled with o-phthalaldehyde under alkaline condition and detected fluorometrically by an FL Detector L-7480 (Hitachi, Tokyo, Japan).

Determination of Histamine content in different parts of airway

After the BALF was collected, tracheas, bronchi and lungs were quickly removed for the determination of histamine content. These tissues were weighed individually. Isolated tissues were homogenized in 0.46 M perchloric acid containing 5 mM Na2-EDTA. The homogenate was centrifuged at 10,000 × g for 15 min at 4 °C and the supernatant was subjected to HPLC-fluorometry.

Statistical analysis

All data were expressed as mean ± SEM. One-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test were used for statistical analyses. P-values less than 0.05 were considered significant.

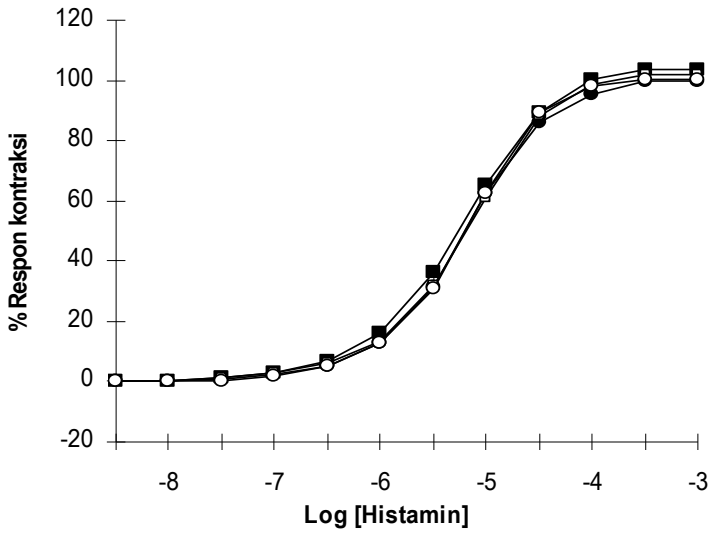

Figure 2. Mean concentration response curve for histamine in absence (●) or in presence of PGV-0 3.10^{-6} M (■); 1.10^{-5} M (□); 3.10^{-5} M (○). Values are mean ± SEM of 4-6 experiments.

Table I. The pD_2 and maximum contraction values of histamine in absence and present of PGV-0

	pD_2	Emax (%)
control	5.22 ± 0.01	100
PGV-0 3.10^{-6} M	5.25 ± 0.08**	103.50 ± 1.19**
PGV-0 1.10^{-5} M	5.20 ± 0.04**	102.15 ± 1.19**
PGV-0 3.10^{-5} M	5.23 ± 0.01**	100.19 ± 2.69**

*, significant different ($P<0.05$) compared to control group
**, not significant different ($P>0.05$) compared to control group

Result and Discussion

In *in vitro* study, histamine obviously stimulated contraction of trachea smooth muscle in concentration-dependent manner. The pD_2 value of histamine that represents a potency of inhibitory effect on the contraction was 5.22 ± 0.01 (Table I). However, the 15-min pre-incubation of guinea-pig trachea with PGV-0 (3.10^{-6}, 1.10^{-5}, 3.10^{-5} M) before histamine addition did not affect the histamine-induced contractions (Fig. 2). The pD_2 and maximum effect of histamine did not change in presence of PGV-0 (Table I).

In *in vivo* study, after last ovalbumin inhalation challenge (provocation step), ovalbumin-sensitized rats showed a significant increase of PenH value observed for 60 minutes (Fig. 3). The maximum increase of PenH could be reached during 20-40 minutes after last ovalbumin challenge (provocation step). It indicates that ovalbumin sensitization followed by challenge and provocation steps can trigger obviously hyperresponsiveness of the airway. Based on this result, the ovalbumin-sensitized rats could be used for animal model of airway hyperresponsiveness.

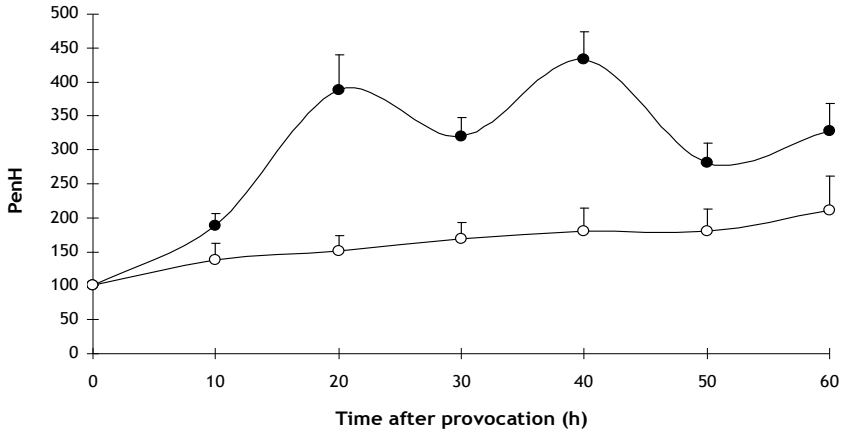

Figure 3. Whole body plethysmograph measurement of airway hyperresponsiveness in rats induced by ovalbumin. After sensitization, the rats were challenged by inhalation of ovalbumin (10 minutes) for the last four days. Airway hyperresponsiveness was characterized by PenH recorded using a whole body plethysmograph measurement system. *, P<0.05 compared to control group (n=5-10). ● = ovalbumin group, ○ = saline group.

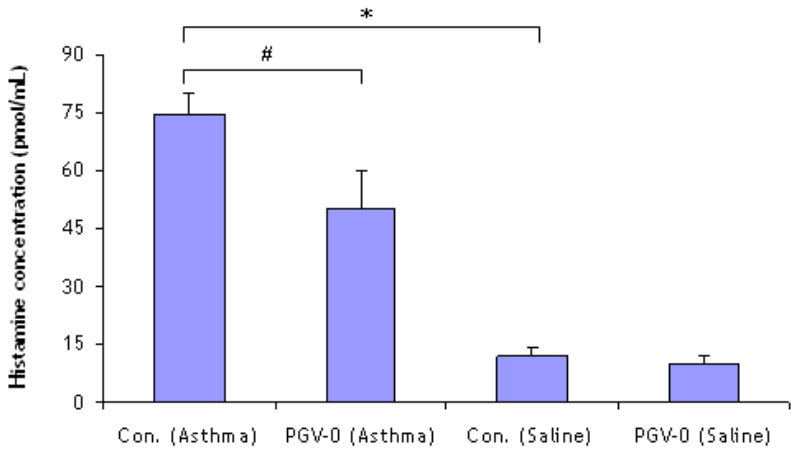

Figure 4. Histamine levels in BALF after last challenge with ovalbumin (asthmatic rats) or saline (normal rats) either in presence or absence of PGV-0 (40 mg/kg BW) (n=3-5). *, P<0.05 compared to control saline group. #, P<0.05 compared to control asthma group.

Multiple exposures of ovalbumin divided into three steps : sensitization, challenge, and final challenge (provocation), trigger allergy inflammatory responses (biphasic response) that are separated by several hours. There is a early-phase airway response (EAR) that is followed by late-phase airway responses (LAR). Histamine has a main role in early-phase airway response. It was used in this study as an allergy inflammatory mediator involved in allergic asthma in rats. In the study, multiple triggers of ovalbumin could increase the histamine

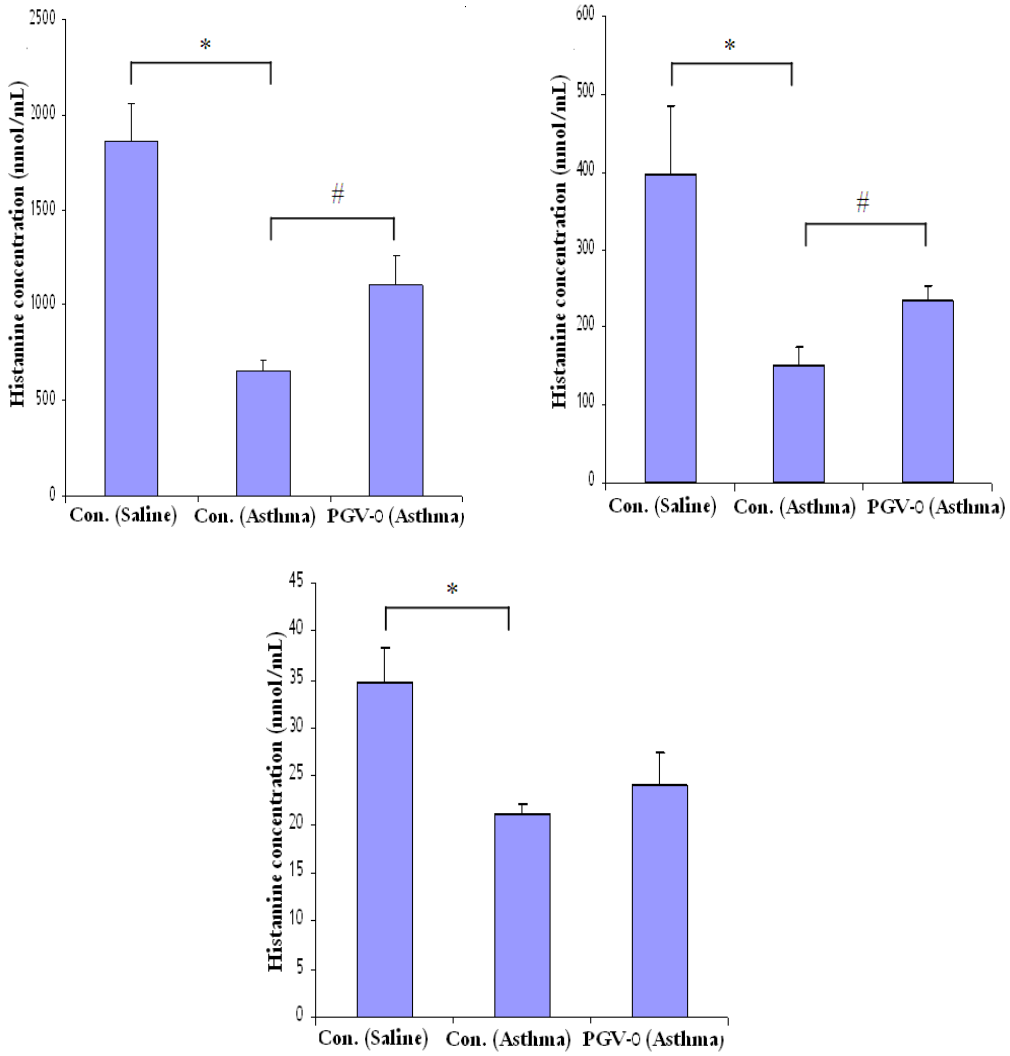

Figure 5. Histamine content after last ovalbumin challenge in trachea (a), bronchus (b), and lung (c) either in presence or absence of PGV-0 (40 mg/kg BW) (n=3-5). *, $P<0.05$ compared to control saline group. #, $P<0.05$ compared to control asthma group.

concentration in bronchoalveolar lavage fluid (BALF) (Fig. 4). Provocation of ovalbumin significantly stimulated histamine in BALF by 74.51±5.33 pmol/mL or 6-times higher than the value of saline administration (control saline group). To determine the preventive effect of curcumin to asthmatic response, rats were orally treated with PGV-0 (40 mg/kg body weight) during sensitization, as described in the methods. The oral administration of PGV-0 significantly decreased ($P<0.05$) the histamine accumulation in BALF to 30 % of the value of control group in asthmatic rats, and did not alter the histamine in normal rats ($P>0.05$).

Figure 5 shows tissue histamine contents in trachea, bronchus, and lung in asthmatic and normal rats. Tissue histamine contents in trachea, bronchus and lung of control saline

group were 1857.4±199.5; 397.1±87.7 and 34.6±3.7 nmol/gram, respectively. After ovalbumin challenge, tissue histamine content significantly decreased to 64.8 % of unchallenged levels (control saline group) in trachea ($P<0.05$), 62.0 % in bronchus ($P<0.05$) and 39.1 % in lung ($P<0.05$). The oral administration of PGV-0 (40 mg/kg BW) significantly prevented the histamine decrease in asthmatic rats to 37.8 % trachea ($P<0.05$), and 34.2 % in bronchus ($P<0.05$). However, PGV-0 did not succeed to prevent the histamine decrease in the lung of asthmatic rats ($P>0.05$).

Asthma is a complex chronic inflammatory airway disease involving multiple pathways and characterized by reversible airway constriction and airway hyperresponsiveness (AHR) to allergens, airway edema, and increased mucus secretion tumor cells. Inhalation challenge of allergic asthmatic with specific allergen evokes a biphasic response separated in the time by several hours. These responses are early-phase airway response (EAR) and late-phase airway responses (LAR). EAR is triggered through allergen-induced crosslinking of specific IgE antibody bound to mast cells through high-affinity receptor FcεRI, and then secrete histamine, cytokines and chemokines. Histamine has a main role in early-phase airway response (Holt *et al.,* 1999; O'Byrne, 1988; Kay, 1991). Indeed, histamine can stimulate contraction of trachea and bronchus smooth muscles by interacting with type 1 histamine receptor (Rang *et al*, 2003). In vitro study, we would like to investigate the effect of PGV-0 to type 1 histamine receptor in isolated guinea pig trachea. The study was conducted using an isolated organ technique with isotonic transducer. The result has shown that PGV-0 could not inhibit the contraction of isolated guinea pig trachea induced by histamine. PGV-0 did not change the pD_2 and Emax values of histamine on trachea smooth muscle. The finding indicates that PGV-0 does not show affinity and intrinsic activity on H-1 histaminergic receptor in trachea smooth muscle.

In contrast, PGV-0 strongly inhibited the histamine release from rat mast cells i.e. RBL-2H3 cells and rat peritoneal mast cells induced by several histamine inducers. Indeed, these effects are more potent than these of curcumin. The mechanism of the inhibitory effects of PGV-0 on histamine release related to blockade of Ca^{2+} signaling events (Nugroho *et al.,* 2009). Moreover, PGV-0 also obviously inhibited the histamine release on compound 48/80-induced rat paw edema (Nugroho *et al.,* 2008). In present study, we studied the effects of PGV-0 on the histamine levels of hiperresponsive airway in allergen asthmatic rats. Multiple triggers of ovalbumin increased the histamine concentration in bronchoalveolar lavage fluid (BALF) (Fig. 3). BALF histamine is released from airway tissues containing mast cells such as trachea, bronchus and lung. PGV-0 (40 mg/kg BW) obviously decreased the histamine level of BALF in asthmatic rats. Besides, PGV-0 significantly prevented the decrease of histamine content in trachea and bronchus, but not in lung. These facts indicate that PGV-0 may prevent mast cells degranulation in airway tissues, and then prevents the histamine release.

In allergic inflammatory asthma, infiltration of eosinophils, neutrophils, macrophages, and lymphocytes into the lumen of airway (trachea, bronchus, and lung) contribute the development of airway inflammation. Inflammatory mediators in the airway are liberated by airways epithelial cells, smooth muscle cells, endothelial and fibroblast. Some of these mediators are IL-3, IL-5 and GM-CSF produced by both Th2 cells and eosinophils, whereas endogenous nitric oxide (NO) produced by inducible NO synthase (Holt *et al.,* 1999; Hogan *et al.,* 1998; Bochner and Busse, 2005). NO strongly promotes the chemotaxis of inflammatory cells in the airway, and supports the development of a Th2 response. Some NO inhibitors were reported can suppress airway inflammation by depleting the recruitment of inflammatory cells, and mucus secretion in the lungs (Robinson *et al.,* 1993; Barnes and Liew, 1995; Barnes *et al.,* 1998, Holt *et al.,* 1999).

Curcumin, a lead compound of PGV-0, inhibited production of cytokines such as IL-5 and IL-8, which are involved in the development of inflammation.

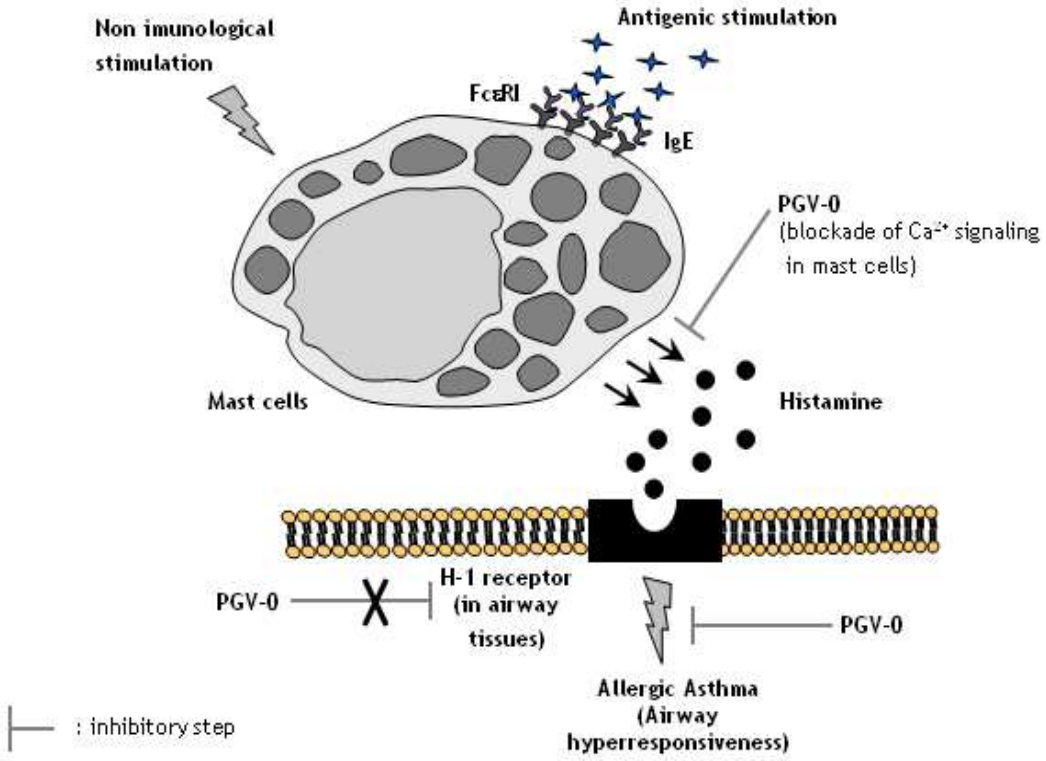

Figure 6. Proposed mechanism of actions of PGV-0 on histamine-mediated hyperresponsive airway.

Besides, curcumin also inhibited the activation of transcription factors like nuclear factor kappa-B (NF- kB) and activating protein 1 (AP-1). Curcumin decreased inducible nitric oxide (iNOS) and NO production induced by IFN-γ in A549 human airway epithelial cells (Moon *et al*., 2008). Moreover, curcumin (20 mg/kg BW) significanly inhibited ovalbumin-induced airway constriction and airway hyperactivity (Ram *et al.,* 2003).

PGV-0 was reported possessing several biological activities such as free radical-related anti-oxidative and anti-cyclooxygenase (Sardjiman, 2000). Besides, free radicals derived from metabolites of unsaturated fatty acid participate to induce the histamine release from mast cells (Di Bello *et al.,* 1998; Mannaioni *et al.,* 1996; Masini *et al.,* 1990).

Another previous study also reported that the inhibitory effect of histamine release of curcumin and its analog was closely related to its antioxidative property (Suzuki *et al.,* 2005). Besides, its anti-inflammatory activity in carrageenan-induced edema experiment was 5-times higher than those of curcumin at dose of 20 mg/kg, po (Sardjiman, 2000). Based on these facts, PGV-0 that possess anti-inflammatory and inhibitory effects on histamine release, can attenuate the impaired airway in asthmatic rats by preventing mast cells degranulation or histamine release from mast, but not influence the interaction between histamine agonis with type 1 histamine receptor (Fig. 6). However, further studies are needed to understand the molecular mechanism of anti-asthmatic effect of PGV-0 in detail.

Conclusion

In vitro study, PGV-0 could not inhibit the contraction of isolated guinea pig trachea induced by histamine. In the other hand, oral administration of PGV-0 (40 mg/kg BW) significantly decreased the histamine accumulation in BALF to 30 % of the value of control group in asthmatic rats. Besides, PGV-0 significantly prevented the histamine decrease in asthmatic rats to 37.8 % trachea, and 34.2 % in bronchus. However, PGV-0 did not succeed to prevent the histamine decrease in the lung of asthmatic rats.

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