

## Inhibitory effect of THPGV-0 on the histamine release from antigen-induced RBL-2H3 cells

### Efek THPGV-0 terhadap pelepasan histamin dari kultur sel RBL-2H3 diinduksi antigen

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#### Abstract

Tetrahydropentagamavunon-0 (THPGV-0) is assumed to be main metabolite product of biotransformation process of PGV-0. THPGV-0 was synthesized by converting PGV-0 to the compound by hydrogenation with Pd/C as a catalyst. PGV-0 potently inhibited the histamine release from rat mast cells *in vitro* and *in vivo*, however, ironically only traces amount of compound was found in the blood. THPGV-0 is assumed to have important roles in the biological effects of PGV-0 *in vivo*. In present study, we investigated the antiallergy effect of THPGV-0 in compare to this of PGV-0 *in vitro*. The study was performed by using rat basophilic leukemia (RBL-2H3) cell line, a tumor analog of mucosal mast cells. DNP-BSA, an antigen, was used as an inducer for stimulating the histamine release from mast cells. In present study, THPGV-0 at low concentration did not succeed to inhibit the histamine release, but at higher concentration (30 and 100  $\mu$ M) showed strong effects. THPGV-0 at concentration of 100  $\mu$ M depleted the histamine release by 96.10  $\pm$ 0.51%. In compare to PGV-0, THPGV-0 has higher efficacy but less potent. In the study, the possibilities of the spontaneous release from RBL-2H3 cells by the compounds were also observed. All concentrations of THPGV-0 as well as PGV-0 showed low spontaneous histamine release, less than 10 % of the total histamine contained in RBL-2H3 cells.

**Key words:** tetrahydropentagamavunon-0, allergy, histamine, RBL-2H3 cells

#### Abstrak

Tetrahidropentagamavunon-0 (THPGV-0) diperkirakan sebagai metabolit utama dari proses metabolisme PGV-0 dalam tubuh. THPGV-0 disintesis melalui hidrogenasi PGV-0 menggunakan katalis Pd/C pada suhu kamar. PGV-0 dapat menghambat pelepasan histamin dari sel mast secara poten pada penelitian *in vitro* maupun *in vivo*. Namun, PGV-0 hanya ditemukan dalam kadar yang sangat rendah dalam darah pada pemberian intravena maupun peroral. THPGV-0 diduga mempunyai peran penting dalam aktivitas biologi PGV-0. Penelitian ini bertujuan untuk mempelajari efek antialergi THPGV-0 dibandingkan dengan PGV-0 secara *in vitro*. Penelitian ini menggunakan kultur sel RBL-2H3 yang kemudian diinduksi oleh DNP-BSA, suatu antigen. Hasil penelitian, THPGV-0 konsentrasi rendah belum mampu menghambat pelepasan histamin, namun pada konsentrasi tinggi (30 and 100  $\mu$ M) menunjukkan efek yang kuat dalam menghambat pelepasan histamin. THPGV-0 100  $\mu$ M secara kuat menghambat pelepasan histamin hingga 96,10  $\pm$ 0,51%. THPGV-0 mempunyai efikasi lebih tinggi dibandingkan PGV-0, namun potensinya lebih rendah. Pada penelitian juga dievaluasi pelepasan histamin spontan dari sel mast. THPGV-0 dan PGV-0 menunjukkan efek pelepasan histamin spontan yang rendah, atau masih lebih rendah dari 10%.

**Kata kunci:** tetrahidropentagamavunon-0, alergi, histamin, RBL-2H3

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## Introduction

2,5-Bis(4-hydroxy-3-methoxybenzylidene)cyclopentanone (Fig. 1), also known as Pentagamavunon-0 (PGV-0), is a benzylidene cyclopentanone analog of curcumin. The compound was firstly synthesized for a number of purposes such the one made by Kodak in 1961 for developing film-forming photosensitive polymers. The compound was reported possessing stronger pharmacological activities such as anti-inflammatory, antioxidative and antibacterial effects in compare to curcumin (Sardjiman, 2000). The compound also showed antiallergy effects in rat basophilic leukemia cell line and rat peritoneal mast cell *in vitro*. PGV-0 inhibited histamine release potently by altering some intracellular signaling events in mast cells, especially by blocking  $Ca^{2+}$  uptake into mast cells (Nugroho *et al.*, 2009). Besides, PGV-0 also inhibited the histamine release from the rat hind paw induced by compound 48/80. PGV-0 markedly decreased the rat paw edema, and prevented the loss of the histamine content and mast cell degranulation in skin tissue (Nugroho *et al.*, 2010a). However, PGV-0 could not inhibit the contraction of isolated guinea pig trachea induced by histamine. PGV-0 significantly decreased the histamine accumulation in BALF to 30 % of the value of control group in asthmatic rats (Nugroho *et al.*, 2010b).

In the study of pharmacokinetics properties of PGV-0 in rats, intravenous injection of the compound at the dose of 20 or 40 mg/kg BW resulted in erratic profile and low levels of the substance in blood. After the injection, the substance levels in blood decreased rapidly during 1-5 min, and followed by a slow elimination process (Nurshanti, 2001). Besides, per oral administration of the compound as well as curcumin also resulted in erratic profile and low levels of the substance in blood. However, the  $C_{max}$  of PGV-0 was higher than this of curcumin (Kustaniah, 2001). The biotransformation of PGV-0 was assumed to be similar to this of curcumin since the chemical structures of these compounds are similar. Curcumin is poorly absorbed in the

intestine. There are facts that the biotransformation of curcumin is fast and could occur during absorption process in gastrointestinal tract. High degree of metabolism of curcumin also occurs in the liver and can be eliminated in the bile rapidly. Its facts suggest that only very low concentration of the substance was found in the body after ingestion. Besides, its metabolites, mainly glucuronated curcumin and tetrahydrocurcumin (THC) serve as the available forms of curcumin *in vivo* (Pan *et al.*, 1999). THC showed equal antiallergy effect in compare to the curcumin. Moreover the THC possessed more potent effects in several biological activities such as antioxidant, antidiabetes, anti-inflammatory. THC has important roles in the biological effects of curcumin (Nugroho, 2006).

In the biotransformation of curcumin, tetrahydrocurcumin is one of the main metabolite of curcumin, and more stable than this of curcumin in buffer solutions of physiologic (pH 7.2) and basic pH at 37 °C (Pan *et al.*, 1999). In addition, tetrahydrocurcumin has equal pharmacological activities in comparison to curcumin (Nugroho, 2006). For elimination process, THC was subsequently glucuronidated by UDP-glucuronosyl transferase yielding THC-glucuronoside (Pan *et al.*, 1999). According to these facts, tetrahydropentagamavunon-0 (THPGV-0) is assumed to be a main metabolite product of biotransformation process of PGV-0 in the body. THPGV-0 was firstly synthesized by Ritmaleni and Simbara (2010). In this study, PGV-0 was converted to THPGV-0 by hydrogenation with Pd/C as a catalyst at room temperature. In present study, we investigated the antiallergy effect of THPGV-0 in comparison to PGV-0 *in vitro*.

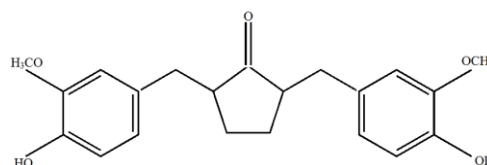


Figure 1. The chemical structure of tetrahydropentagamavunon-0 (THPGV-0).

## Methodology

### Materials

Tetrahydropentagamavunon-0 (THPGV-0) was synthesized through two steps. Firstly, pentagamavunon-0 (PGV-0) was synthesized by reacting vanillin and cyclopentanone in acidic condition. Second step, the compound was converted to THPGV-0 by hydrogenation with Pd/C as a catalyst at room temperature (Ritmaleni and Simbara, 2010). DNP<sub>24</sub>-BSA as an antigen and monoclonal IgE against DNP<sub>24</sub>-BSA purified from supernatant in IgE producing hybridoma, were produced in Department of Pharmacology, School of Medicine Ehime University Japan. Eagle's minimum essential medium (MEM) and antibiotics (combination of penicillin G sodium and streptomycin sulfate) were purchased from Gibco (Grand Island, NY, USA). Fetal calf serum was obtained from JRH Biosciences (Kansas, USA). Piperazine-1,4-bis(2-ethanesulfonic acid) (PIPES) was purchased from Dojindo (Kumamoto, Japan), and *o*-phthalaldehyde was from Wako Pure Chemical Industries. (Osaka, Japan).

### Culture of RBL-2H3 cells

The cells were cultured in MEM containing 15% fetal calf serum and antibiotics (penicillin and streptomycin) in a flask in a humidified atmosphere (5% CO<sub>2</sub>) at 37 °C as described by Barsumian *et al.* (1981). For the assay of histamine release, cells were seeded into 24-well culture plates at a density of 5 x 10<sup>5</sup> cells/0.4 ml per each well. The cells were incubated overnight at 37 °C. For DNP<sub>24</sub>-BSA experiments, the cells were sensitized with 0.5 µg/mL of monoclonal IgE. On the second day, the medium was removed, and the cells were washed twice with 500 µL of PIPES buffer (119mM NaCl, 5mM KCl, 25mM PIPES, 5.6mM glucose, 0.4mM MgCl<sub>2</sub>, 1mM CaCl<sub>2</sub>, 40mM NaOH, and 0.1 % BSA, pH 7.2), and preincubated for 10 min at 37 °C after addition of 180 µL PIPES buffer either without (as a negative control) or with the drug. After 10 min preincubation, 20 µL of stimulant (200 ng/mL DNP<sub>24</sub>-BSA, 5 µM thapsigargin, or 10 µM ionomycin) were added to each well and the plate was incubated at 37°C for 30 min.

### Assay of histamine release

Histamine released in the medium was measured by HPLC-fluorometry according to Yamatodani *et al.* (1985). After a 30 min incubation, the plates were centrifuged at 1,800 x g for 5 min and 50 µL of the supernatant was mixed with 250 µL of 3% perchloric acid containing 5mM Na<sub>2</sub>-EDTA. After addition of 30 µL of 2 M KOH/1 M

KH<sub>2</sub>PO<sub>4</sub> and centrifugation at 10,000 x g for 15 min at 4°C, 50 µL of the supernatant was injected directly onto a column packed with TSKgel SP-2SW cation exchanger (Tosoh, Tokyo). For measuring the total histamine content in cells, 350 µL of PIPES buffer was added to 6 wells and the cells were then sonicated. Fifty microlitres cell homogenate was used for the histamine assay described above. Histamine was eluted with 0.25 M potassium phosphate at a flow rate of 0.6 mL/min, and post-labeled with *o*-phthalaldehyde under alkaline conditions and detected using a F1080 Fluorometer (Hitachi, Tokyo) at excitation and emission wavelengths of 360 and 450 nm, respectively.

### Data analysis

The values were expressed as a percentage of net histamine release. The percent inhibition of histamine release was calculated according to the following equation: inhibition of histamine release (%) = [(histamine release in the absence of the test compound - histamine release in the presence of the test compound) / histamine release in the absence of the test compound] x 100. Spontaneous release (%) = (histamine content in the supernatant of unstimulated cells/total histamine content) x 100.

The IC<sub>50</sub> values are concentration of the compounds causing half maximal inhibitory effects on the histamine release from mast cells. 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.

## Result and Discussion

Dinitrophenylated bovine serum albumin (DNP-BSA), an antigen was used to induce the histamine release from RBL-2H3 cells, a tumor analog of mucosal mast cells. In the experiment, we evaluated the effects of various concentration of DNP-BSA on the histamine release. DNP-BSA could stimulate the histamine release in concentration-dependent manner, even though at higher concentration tend to show lower effect as showed at concentration of 200 ng/mL. In the study, DNP-BSA at the concentration of 20 ng/mL has showed a maximum effect by stimulating the histamine release by 33.07±2.79 % (n=3). Based on this result, concentration of 20 ng/mL was used for inducing the histamine release in the next experiments.

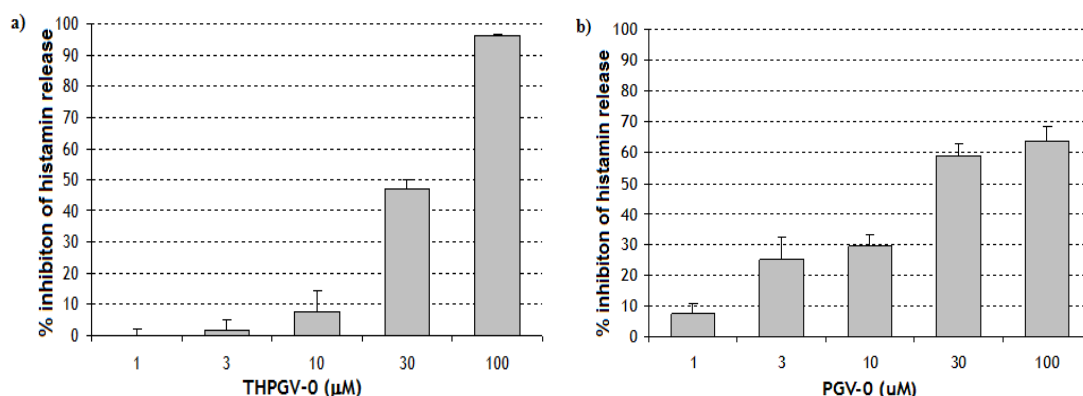


Figure 2. Effects of various concentrations (1-100 μM) of tetrahydropentagamavunon-0 (THPGV-0) (a) or pentagamavunon-0 (PGV-0) (b) on the histamine release from RBL-2H3 cells induced by DNP-BSA 20 ng/mL. The data were representative of three-four independent experiments.

Table I. Spontaneous histamine release from RBL-2H3 cells by THPGV-0 in absence of the antigen. (n = 3 – 4)

Concentration (μM) of the compound	Spontaneous histamine release (%)	
	THPGV-0	PGV-0
1	1.05±2.97	2.56±2.21
3	1.13±2.10	2.96±2.85
10	1.21±2.12	6.40±3.20
30	6.13±7.12	8.62±0.49
100	6.84±3.40	9.01±0.63
(Thapsigargin 0.5 μM)	74.30±7.10	

In the study, THPGV-0 showed a gradual inhibitory effect on histamine release from RBL-2H3 cells (Fig. 2a). The effect of THPGV-0 was concentration-dependent. At lower concentrations, THPGV-0 showed slight inhibitory effects on the histamine release ( $P>0.05$ ). In contrast, at higher concentrations (30 and 100 μM) the inhibitory effects of THPGV-0 increased progressively ( $P<0.05$ ). Moreover, at concentration of 100 μM, THPGV-0 strongly depleted the histamine release from RBL-2H3 cells by  $96.10 \pm 0.51\%$ . The  $IC_{50}$  value of THPGV-0 was 34.10 μM. The maximum inhibitory effect ( $E_{max}$ ) and  $IC_{50}$  values represent efficacy and potency of the compound, respectively.

In the other side, PGV-0 also showed gradual inhibitory effects on histamine release from RBL-2H3 cells (Fig. 2b). The effect of PGV-0 was concentration-dependent. PGV-0 obviously showed the inhibitory effect started at concentration of 3 μM ( $P<0.05$ ). At concentration of 100 μM, PGV-0 suppressed the histamine release from RBL-2H3 cells by  $63.66 \pm 4.68\%$ . The  $IC_{50}$  value of PGV-0 was 23.79 μM.

We also observed the possibility of the spontaneous release from RBL-2H3 cells by THPGV-0 with a series of concentration (1-100 μM). The effect was considered significant if THPGV-0 caused spontaneous histamine release of more than 10 %. All

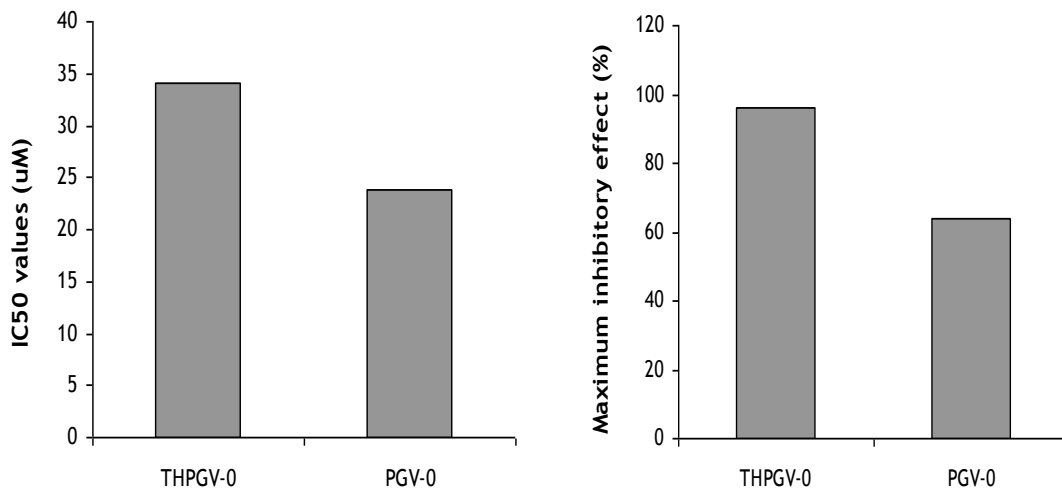


Figure 3. Comparison between the IC<sub>50</sub> and E<sub>max</sub> values of THPGV-0 with PGV-0. Both values represent potency and efficacy of the inhibitory effects of THPGV-0 and PGV-0.

concentrations of THPGV-0 as well as PGV-0 showed low spontaneous histamine release, less than 10 % of the total histamine contained in RBL-2H3 cells (Table I). As a comparison, thapsigargin, a sesquiterpene lactone isolated from the plant *Thapsia garginica* (Patkar *et al.*, 1979), potently stimulated the histamine release from RBL-2H3 cells by 74.3±7.1%.

Allergy reaction can be triggered by allergens, such as grass pollen, dust mite, certain foodstuffs or some drugs, that evoke the production of IgE type then attach mast cell via the high-affinity FcεRI receptors on the mast cell surface (Rang *et al.*, 2003). DNP<sub>24</sub>-BSA is a specific antigen for monoclonal IgE antibody. The antigen cross-link into IgE antibody molecules on FcεRI receptors to generate a series of intracellular signaling in mast cells. Finally, these phenomena trigger the granule exocytosis releasing histamine from mast cells (Metcalf *et al.* 1997; Liu *et al.*, 1980).

In the case of curcumin, tetrahydrocurcumin (THC) serves as the available form of curcumin *in vivo*. Tetrahydrocurcumin is one of the main metabolite of curcumin, and more stable than this of curcumin in buffer solutions of physiologic (pH 7.2) and basic pH. at 37°C

(Pan *et al.*, 1999). THC showed an equal antiallergy effect in comparison to the curcumin. Moreover the THC possessed more potent effects in several biological activities such as antioxidant, antidiabetes, anti-inflammatory. THC has important roles in the biological effects of curcumin (Nugroho, 2006). For elimination process, THC was subsequently glucuronidated by UDP-glucuronosyl transferase yielding THC-glucuronoside (Pan *et al.*, 1999). THPGV-0 is assumed to be a main metabolite of PGV-0. In the study of pharmacokinetics properties of PGV-0 in rats, the substance levels in blood decreased rapidly during 1-5 min, and followed by a slow elimination process after intravenous injection (Nurshanti, 2001). In addition, per oral administration of the compound as well as curcumin also resulted in erratic profile and low levels of the substance in blood (Kustaniah, 2001). Sugiyanto *et al.* (2003) reported that PGV-0 underwent phase 1 biotransformation, and produced at least one metabolite. One metabolite was suspected as tetrahydropentagamavunon-0 (THPGV-0). Subsequently, the metabolites still possessing hydroxyl group underwent conjugation process (phase 2 biotransformation).

*In vivo* study, PGV-0 potently inhibited the histamine release from the rat hind paw induced by compound 48/80. In addition, PGV-0 could decrease the rat paw edema, and prevented the loss of the histamine content and mast cell degranulation in skin tissue (Nugroho *et al.*, 2010<sup>a</sup>). PGV-0 did not influence the contraction of isolated guinea pig trachea induced by histamine, however, potently decreased the histamine accumulation in BALF to 30 % of the value of control group in asthmatic rats. In addition, 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 (Nugroho *et al.*, 2010<sup>b</sup>). PGV-0 showed potent pharmacological effects *in vivo* such as anti-inflammatory and antiallergy, but it poorly was found in the blood. These facts indicate that THPGV-0 might have important roles in the biological effects of PGV-0 *in vivo*.

There are only few studies about the pharmacological activities of THPGV-0. Agustina (2010) reported that THPGV-0 showed antifungal activity on *C. albicans*. Simbara (2009) reported that the compound as well as PGV-0 showed DPPH free radical scavenger activity *in vitro*. The antioxidative activity of THPGV-0 on DPPH free radical was more potent than this of PGV-0 or vitamin E. In the case of tetrahydrocurcumin, its aromatic or phenolic hydroxy and  $\beta$ -diketon moieties are assumed to have important roles in its antioxidative property (Sugiyama *et al.*, 1996; Priyadarsini *et al.*, 2003). Suzuki *et al.* (2005) reported that the inhibitory effect of curcumin and its analogues on histamine release were closely related to its antioxidant properties. Free radicals derived from metabolites of unsaturated fatty acids participate in the induction of histamine release (Di Bello *et al.*, 1998; Mannaioni *et al.*, 1996). In present study, THPGV-0 at concentration of

100  $\mu$ M strongly depleted the histamine release from RBL-2H3 cells a tumor analog of mucosal mast cells, by  $96.10 \pm 0.51\%$ . As a comparison, PGV-0 at the concentration of 100  $\mu$ M inhibited the histamine release by  $63.66 \pm 4.68\%$ . Maximum inhibitory effect of THPGV-0 is higher than this of PGV-0. These facts indicate THPGV-0 is stronger than PGV-0 to inhibit the histamine release. However, the  $IC_{50}$  of THPGV-0 was higher than this of PGV-0 so the potency of THPGV-0 was less than this of PGV-0 (Fig. 3). These facts indicate that THPGV-0 has higher efficacy than this of PGV-0, however, THPGV-0 was less potent than this of PGV-0. Efficacy is capacity to produce a maximum effect, whereas potency is a function of drug concentration required for its specific effect (usually half of maximal effects) to occur. Modification of the chemical structure of PGV-0 due to its biotransformation process was suggested to influence its potency and efficacy. However, further investigation is needed to explain these phenomena.

In the previous study, benzylidenecyclopentanone curcumin analogues including PGV-0 were investigated for their effects on viability of RBL-2H3 for certain time incubation of the compounds. In the case of PGV-0, treatment with various concentrations of the compound (1, 10, or 100  $\mu$ M) for 30 min did not influence the viability of RBL-2H3 cells, however, they markedly decreased the viability of the cells after 24 hours incubation in dose-dependent manner (Nugroho *et al.*, 2010<sup>c</sup>).

## Conclusion

THPGV-0 succeeded to inhibit the histamine release from RBL-2H3 cells induced by DNP-BSA. THPGV-0 has higher efficacy than this of PGV-0, however, THPGV-0 was less potent than this of PGV-0.

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