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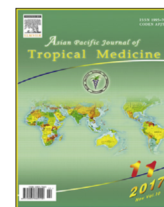

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Effects of 8-Hydroxyisocapnolactone-2-3-diol and friedelin on mast cell degranulation

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ABSTRACT

Objective: To investigate the effects of friedelin (terpenoid) and 8-hydroxyisocapnolactone-2-3-diol (coumarin) with concentration 10 μM, 30 μM, and 100 μM on inhibiting mast cells (MCs) degranulation.

Methods: The investigation was performed *in vitro* by administering each compound into rat peritoneal MCs and rat basophilic leukemia-2H3 cells followed by activation with 50 μg/mL of compound 48/80 or 1 μM of ionomycin. The concentration of histamine released from each group was measured by a high-performance liquid chromatography-fluorometry system with post-column derivatization using o-phthalaldehyde.

Results: 8-Hydroxyisocapnolactone-2-3-diol inhibited degranulation of compound 48/80 activated-rat peritoneal MCs with the histamine release percentages of 74.57%, 72.21% and 51.79% when the 10 μM, 30 μM and 100 μM concentrations were used, respectively. Where as about 81% histamine was released by the control group. Degranulation inhibition ability was also observed in ionomycin-activated rat basophilic leukemia-2H3 cells. In contrast, friedelin failed to inhibit degranulation in either cell type. The inhibition of 8-hydroxyisocapnolactone-2-3-diol was not related to the depletion of histamine synthesis as implied by the total histamine measurement.

Conclusions: These results exhibit the promising of 8-hydroxyisocapnolactone-2-3-diol is a potential parent structure for developing a MCs stabilizer.

1. Introduction

Natural products either as a new drug entities or as a building blocks are still the major sources of drugs we used today [1]. These products are usually the secondary metabolites of from broad type of organisms (plants, animal, *etc.*) and have very diverse chemical structures [2]. All of these compounds are widely used as drugs of choice for several kinds of diseases with diverse proposed mechanisms [1,3]. Statins, a class of cholesterol lowering drug, is one of block buster drugs that were obtained from isolating natural products or a synthetic based on the original natural product structure [4]. Compounds from natural products [5,6] or synthetic drugs based on a natural product structure, such as curcumin derivatives [6,7] have been tested on allergic conditions with promising results.

Friedelin, a terpenoid constituent and plant secondary metabolite, is found in several tropical plants *i.e.*, *Anchietia salutaris* var. *martiana* [5], *Azima tetracantha* Lam [8], and a subtropical plant in Poland and Finland i.e., *Vaccinium vitis-idaea* [9]. This compound has been reported to act as an anti-inflammatory and analgesic in mice and rats [8], and also as a histamine H1 receptor (H1R) antagonist [10]. 8-Hydroxyisocapnolactone-2-3-diol is a coumarin isolated from *Eugenia chloranta* Duthie and *Micromelum minutum* [11] that exhibits cytotoxicity against Leishmania major and several cancer cell lines [12]. The effects of friedelin and 8-hydroxyisocapnolactone-2-3-diol on mast cells (MCs) have not been studied.

MCs are known for their main role in modulating allergic responses, and are also involved in the defense against parasites and the inflammatory pathogenesis [13]. These cells contain intracellular granules that store histamine, cytokines, proteases and other mediators that orchestrate allergic and inflammatory conditions [13,14]. After activation, MCs release their granules

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in a process called degranulation and expose all of their contents to the extracellular environment [14]. The release of MCs granule content and the immediately synthesized compounds is a crucial stage in the allergic and inflammation pathogenesis [13,14]. The release of histamine or β-hexosaminidase is often used to determined the state of MCs [15], such as whether they are in a basal or in an activated condition.

In the present study, we investigated the activities of friedelin and 8-hydroxyisocapnolactone-2-3-diol for inhibiting MCs degranulation. Rat peritoneal mast cells (RPMCs) are connective tissue MCs that respond to antigen, basic compound and ionophore stimulation [13]. Rat basophilic leukemia-2H3 (RBL-2H3) cells are a type of mucosal MCs and could only respond to antigen and ionophore stimulation [13]. Of the two compounds, only 8-hydroxyisocapnolactone-2-3-diol shows potency to stabilize both MCs after activation with compound 48/80 or ionomycin.

2. Materials and methods

2.1. Reagents and chemicals

O-phthalaldehyde, H_3PO_4, diethyl ether, NaCl, KCl, $Na_2HPO_4 \cdot H_2O$, KH_2PO_4, NaOH, perchloric acid, glucose, $MgCl_2$, and $CaCl_2$ were purchased from Wako Pure Chemical Co., (Osaka, Japan). Fetal bovine serum (FBS) was obtained from JRH Bioscience (Lenexa, KS, USA). Antibiotic (5 000 units/mL penicillin and 5 000 μg/mL streptomycin) and Eagle's Minimum Essential Medium (MEM) were purchased from Gibco, Life Technologies (Grand Island, NY, USA). Bovine serum albumin (BSA), ionomycin, compound 48/80, and Percoll were obtained from Sigma–Aldrich (St Louis, MO, USA). Heparin obtained from Mochida Pharmaceutical (Tokyo, Japan), friedelin and 8-hydroxyisocapnolactone-2-3-diol were prepared in our laboratory. The PIPES buffer used in this experiment contained 25.0 mM of PIPES (Dojindo, Kumamoto, Japan), 119.0 mM NaCl, 5.0 mM KCl, 5.6 mM glucose, 0.4 mM $MgCl_2$, 40.0 mM NaOH, and 0.1% BSA. The phosphate buffered saline (PBS) used to collect the RPMCs consisted of 137.0 mM NaCl, 2.7 mM KCl, 10.0 mM $Na_2HPO_4 \cdot H_2O$, and 1.76 mM KH_2PO_4.

2.2. RPMC isolation

Six-month-old Wistar rats were killed with diethyl ether, followed by a 20 mL *i.p* injection of heparin-containing PBS. The abdominal region was massaged for 2 min, and the intraperitoneal solution (previously injected PBS) was collected. The solution was centrifuged for 5 min at 1 000 rpm and 4 °C, the supernatant was discarded and the cell pellet was resuspended in 2 mL of PBS. The cells suspension was loaded onto 4 mL of 60% Percoll solution and centrifuged for 20 min at 500 rpm and 4 °C. The Percoll solution was discarded, and the cell pellet was washed with PBS solution. The cell pellet was resuspended in PIPES buffer, stained with toluidine blue solution (Wako Pure Chemical Co., Osaka, Japan), and the number of cells was counted. All of the PBS solutions used in the experiment contained 0.1% BSA.

2.3. RPMC histamine release assay

Suspension of RPMCs in PIPES buffer were added to a 96 well plate (180 μL/well) at a density of 2 000–5 000 cells/mL. After adding 20 μL of friedelin or 8-hydroxyisocapnolactone-2-3-diol (final concentrations: 10 μM, 30 μM and 100 μM), the RPMCs were incubated for 10 min at 37 °C in a waterbath shaker. Then, 2 μL of compound 48/80 (final concentration, 50 μg/mL) was added and the incubation was continued for 15 min. Furthermore, the plate was spun at 1 000×*g* for 5 min, at room temperature, 50 μL of supernatant was collected and mixed with 250 μL of 2.5% perchloric acid. Finally, 1 M KH_2PO_4/2 M KOH was added and the mixture was centrifuged at 10 000×*g* and 4 °C for 5 min. Histamine was measured in the supernatant using a high-performance liquid chromatography-fluorometric method [16].

To measure total histamine content, the remaining 150 μL of solution in the wells was sonicated. Another 50 μL of the solution from a representative treatment was treated as described above. As RPMCs used in this experiment were freshly isolated primary cells, the condition of the cells could be varied depending on the isolation method and condition. To ensure the collected RPMCs were in a proper condition to be used for this experiment, their response toward compound 48/80 activation were characterized and stained with toluidine blue dye which would bind to intragranular proteoglycan.

2.4. RBL-2H3 cell histamine release assay

RBL-2H3 cells were passage in MEM contained 1% antibiotics and 15% FBS. The RBL-2H3 cells in MEM was distributed to a 24 well plate (400 μL/well) with density 5×10^5 cells/mL and then incubated overnight at 37 °C with 5% CO_2. The next day, MEM medium was discarded, and 200 μL pipes buffer containing friedelin or 8-hydroxyisocapnolactone-2-3-diol was added with various concentration as stated above. After incubation for 10 min at 37 °C on waterbath shaker, 2 μL of ionomycin (final concentration 1 μL) was added and incubated for 15 min. Here after, the sample for histamine measurement was prepared as state above.

2.5. Data analysis

All data are presents as mean ± SEM of triplicate experiments. The statistical analysis was done using the two samples *t*-test assuming unequal variance in Microsoft Excel with $P < 0.05$ or $P < 0.01$ were considered as statistically significant difference.

3. Results

3.1. 8-Hydroxyisocapnolactone-2-3-diol inhibits compound 48/80 induced-histamine release

Compound 48/80 treatment induced degranulation with histamine release level rose up to around 60%, and in the control group the spontaneous release was very low (1.14% ± 0.09%). Toluidine blue staining showed that most of the cells collected stained with strong blue color which is the characteristic of RPMCs. The granules could also be seen under the light microscope. However, there are cells that stained only with pale blue or cells with smaller size, which belong to other peritoneal cells. The results confirmed the RPMC isolation protocol was not harmful to the cells and the cells could be collected in high purity.

Table 1

Effect of friedelin and 8-hydroxyisocapnolactone-2-3-diol on histamine release and total histamine content in compound 48/80 (50 μg/mL)-activated RPMCs.

Compound	Concentrations (μM)	Histamine release (%)	Total histamine content (%)
Friedelin	0	79.65 ± 0.86	100.00 ± 2.24
	10	86.02 ± 1.18*	98.74 ± 2.94
	30	87.25 ± 1.33*	131.08 ± 27.00
	100	86.69 ± 1.28*	102.42 ± 1.64
8-Hydroxyisocapnolactone-2-3-diol	0	81.19 ± 1.79	100.00 ± 1.72
	10	74.57 ± 2.08	103.28 ± 1.91
	30	72.21 ± 0.36*	103.96 ± 3.43
	100	51.79 ± 2.42**	108.29 ± 3.30

*$P < 0.05$ and **$P < 0.01$ compared to control.

Table 2

Effect of friedelin and 8-hydroxyisocapnolactone-2-3-diol on histamine release and total histamine content in ionomycin (1 μM)-activated RBL-2H3 cells.

Compound	Concentrations (μM)	Histamine release (%)	Total histamine content (%)
Friedelin	0	50.19 ± 2.33	100.00 ± 0.59
	10	49.64 ± 1.98	104.28 ± 1.70
	30	44.31 ± 3.86	99.26 ± 1.66
	100	51.54 ± 2.41	99.58 ± 1.39
8-Hydroxyisocapnolactone-2-3-diol	0	55.73 ± 1.40	100.00 ± 8.31
	10	51.92 ± 2.11	97.37 ± 2.57
	30	49.49 ± 1.41*	96.04 ± 0.62
	100	42.10 ± 1.27**	92.66 ± 1.69

*$P < 0.05$ and **$P < 0.01$ compared to control.

In the present experiment, the potency of friedelin (terpenoid) and 8-hydroxyisocapnolactone-2-3-diol (coumarin) for inhibiting MCs activation was investigated. Friedelin concentrations of 10 μM, 30 μM and 100 μM failed to inhibit degranulation after compound 48/80 induced-RPMCs activation (Table 1). In contrast, histamine release increased significantly compared to the control group and the increase was about 10% for all of the friedelin doses. When the same concentration range (10 μM, 30 μM and 100 μM) of 8-hydroxyisocapnolactone-2-3-diol was tested on RPMCs, degranulation triggered by compound 48/80 (50 μg/mL) administration was suppressed in a dose-dependent manner. Histamine release decreased from 81.19% in the control group to 74.57%, 72.21% and 51.79% in the groups receiving 10 μM, 30 μM and 100 μM of 8-hydroxyisocapnolactone-2-3-diol, respectively (Table 1). These results indicate that only high concentrations (30 μM and 100 μM) of 8-hydroxyisocapnolactone-2-3-diol significantly decreased histamine release compared to that in the control group (Table 1).

3.2. 8-Hydroxyisocapnolactone-2-3-diol inhibits ionomycin induced-histamine release

Moreover, when RBL-2H3 cells were used instead of RPMCs, an identical data pattern was obtained. Friedelin was ineffective at inhibiting MC degranulation triggered by the administrating 1 μM of ionomycin (Table 2). Different with in the compound 48/80-induced RPMCs, the level of histamine release in ionomycin-induced RBL-2H3 cells all concentration group was comparable with control group and did not show any increasing pattern. On the other hand, 8-hydroxyisocapnolactone-2-3-diol showed a dose dependent degranulation suppression with the highest response was obtained at concentration 100 μM (Table 2). Histamine release in the control group was 55.73%, where as in the group with 10 μM, 30 μM and 100 μM 8-hydroxyisocapnolactone-2-3-diol were 51.92%, 49.49% and 42.10%, respectively. The degranulation suppression ability of 8-hydroxyisocapnolactone-2-3-diol in RPMCs and RBL-2H3 cells was not accompanied by a reduction in histamine synthesis as depicted in the total histamine content data in Tables 1 and 2. Friedelin treatment also did not affect the total histamine content in both cells (Tables 1 and 2).

4. Discussion

Stabilization of MCs has been extensively studied because of the importance of activating MCs for the allergic and inflammatory responses. Many synthetic and natural compounds have been tested for their ability to inhibit MCs activation [6]. In the present report, we show that the naturally occurring coumarin, 8-hydroxyisocapnolactone-2-3-diol inhibited degranulation of MCs triggered by ionomycin or compound 48/80.

Degranulation of MCs can be triggered by a wide variety of secretagogues, from small chemical molecules to large proteins, by different activation pathways [13]. Here ionomycin and compound 48/80 were used, as they represent two different pathways of MC degranulation. Ionomycin is a polyether antibiotic [17] and its interaction with Ca^{2+} helps the ion enter the cell causing an increase in intracellular Ca^{2+} concentration [18]. The increase in intracellular Ca^{2+} concentration will triggers the degranulation process. On the other hand, compound 48/80 induces MC degranulation by binding to its receptor on the plasma membrane which further triggers the intracellular signaling including increases in intracellular Ca^{2+} concentration [19]. Our results showed that 8-hydroxyisocapnolactone-2-3-diol inhibited both of these pathways, which is in accordance with a

previous report showing that an α,β-unsaturated lactone (structure of 8-hydroxyisocapnolactone-2-3-diol) inhibited compound 48/80-induced RPMC activation [20]. How 8-hydroxyisocapnolactone-2-3-diol suppressed degranulation remains unclear. However, it is plausible that 8-hydroxyisocapnolactone-2-3-diol suppressed degranulation through by inhibiting protein phosphorylation which is an important step in intracellular signaling. A previous report explained that coumarin can act as protein phosphorylation inhibitor [21,22].

Another compound that where investigated in the present report, friedelin terpenoid, was failed to exhibited any degranulation inhibition both in ionomycin- and compound 48/80-activated cells. This was an interesting finding as a previous study reported that friedelin inhibits histamine-induce guinea pig tracheal contraction by competitively antagonizing the H1R [10]. In another report, it was also explained that some terpenoids exhibiting MCs stabilization activity [6]. Therefore, we hypothesized that friedelin could also suppress histamine release from MCs. Nevertheless, in our study friedelin did not antagonized Mrgpr or disturbed the intracellular signaling triggered by compound 48/80 or ionomycin. These results are in accordance with the study by Di Stasi and friends where friedelin is inactive in inhibiting histamine release from guinea pig lung cells [5]. Our data showed the ability of friedelin to increase the histamine release from compound 48/80-induced RPMCs. This was also interesting, as friedelin did not increase histamine release from RBL-2H3 cells, and friedelin did not augment histamine production in RPMCs or RBL-2H3 cells. Future research is needed to confirm this phenomena and the molecular mechanism of inhibiting MC degranulation by 8-hydroxyisocapnolactone-2-3-diol.

Taken together, our results show that 8-hydroxyisocapnolactone-2-3-diol has the ability to suppress degranulation induced by ionomycin and compound 48/80, whereas friedelin failed to show the same effects. Therefore, the 8-hydroxyisocapnolactone-2-3-diol structure may be useful as a parent structure for developing a MC stabilizer.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Newman DJ, Cragg GM. Natural products as sources of new drugs from 1981 to 2014. *J Nat Prod* 2016; **79**(3): 629-661.

[2] Dias DA, Urban S, Roessner U. A historical overview of natural products in drug discovery. *Metabolites* 2012; **2**(2): 303-336.

[3] Angulo P, Kaushik G, Subramaniam D, Dandawate P, Neville K, Chastain K, et al. Natural compounds targeting major cell signaling pathways: a novel paradigm for osteosarcoma therapy. *J Hematol Oncol* 2017; **10**(1): 10.

[4] Stancu C, Sima A. Statin: mechanism of action and effects. *J Cell Mol Med* 2001; **5**(4): 378-387.

[5] Di Stasi LC, Gomes JC, Vilegas W. Studies on anti-allergic constituents in the leaves and stems of *Anchietia salutaris* var. *martiana* (Violaceae). *Chem Pharm Bull* 1999; **47**(6): 890-893.

[6] Finn DF, Walsh JJ. Twenty-first century mast cell stabilizers. *Br J Pharmacol* 2013; **170**(1): 23-37.

[7] Nugroho AE, Ikawati Z, Sardjiman, Maeyama K. Effects of benzylidenecyclopentanone analogues of curcumin on histamine release from mast cells. *Biol Pharm Bull* 2009; **32**(5): 842-849.

[8] Antonisamy P, Duraipandiyan V, Ignacimuthu S. Anti-inflammatory, analgesic and antipyretic effects of friedelin isolated from *Azima tetracantha* Lam. in mouse and rat models. *J Pharm Pharmacol* 2011; **63**(8): 1070-1077.

[9] Szakiel A, Paczkowski C, Koivuniemi H, Huttunen S. Comparison of the triterpenoid content of berries and leaves of lingonberry *Vaccinium vitis-idaea* from Finland and Poland. *J Agric Food Chem* 2012; **60**(19): 4994-5002.

[10] Nugroho AE, Susidarti RA, Astuti P. Effects of friedelin from *Eugenia chlorantha* Duthie on physiological receptors-operated Guinea-pig trachea contraction. *JPRCP* 2011; **1**(2): 71-78.

[11] Rahmani M, Susidarti RA, Ismail HBM, Sukari MA, Hin TYY, Lian GEC, et al. Coumarins from Malaysian *Micromelum minutum*. *Phytochemistry* 2003; **64**(4): 873-877.

[12] Sakunpak A, Matsunami K, Otsuka H, Panichayupakaranant P. Isolation of new monoterpene coumarins from *Micromelum minutum* leaves and their cytotoxic activity against *Leishmania major* and cancer cells. *Food Chem* 2013; **139**(1–4): 458-463.

[13] Metcalfe DD, Baram D, Mekori YA. Mast cells. *Physiol Rev* 1997; **77**(4): 1033-1079.

[14] Moon TC, Befus AD, Kulka M. Mast cell mediators: their differential release and the secretory pathways involved. *Front Immunol* 2014; **5**: 569.

[15] Larson D, Mitre E. Histamine release and surface CD200R1 staining as sensitive methods for assessing murine mast cell activation. *J Immunol Methods* 2012; **379**(1–2): 15-22.

[16] Yamatodani A, Fukuda H, Iwaeda T, Watanabe T, Wada H. HPLC determination of plasma and brain histamine without previous purification of biological samples: cation exchange chromatography coupled with post-column derivatization fluorometry. *J Chromatogr* 1985; **344**: 115-123.

[17] Evans DA, Dow RL, Shih TL, Takacs JM, Zahler R. Total synthesis of the polyether antibiotic ionomycin. *J Am Chem Soc* 1990; **112**(13): 5290-5313.

[18] Morgan AJ, Jacob R. Ionomycin enhances Ca^{2+} influx by stimulating store-regulated cation entry and not by a direct action at the plasma membrane. *Biochem J* 1994; **300**(Pt 3): 665-672.

[19] Grimbaldeston MA. Mast cell-MrgprB2: sensing secretagogues or a means to overreact? *Immunol Cell Biol* 2015; **93**(3): 221-223.

[20] Penissi AB, Vera ME, Mariani ML, Rudolph MI, Cenal JP, de Rosas JC, et al. Novel anti-ulcer α,β-unsaturated lactones inhibit compound 48/80-induced mast cell degranulation. *Eur J Pharmacol* 2009; **612**(1–3): 122-130.

[21] Yang EB, Zhao YN, Zhang K, Mack P. Daphnetin, one coumarin derivatives, is a protein kinase inhibitor. *Biochem Biophys Res Comm* 1999; **260**(3): 682-685.

[22] Drabikova K, Perecko T, Nosal R, Harmatha J, Smidrkal J, Jancinova V. Study of possible mechanisms involved in the inhibitory effects of coumarin derivatives on neutrophil activity. *Oxid Med Cell Longev* 2013; **2013**: 136570.