



Structure–activity relationships of vanillic acid ester analogs in inhibitory effect of antigen-mediated degranulation in rat basophilic leukemia RBL-2H3 cells



Nao Ishimata^a, Hideyuki Ito^b, Akihiro Tai^{a,*}

^a Faculty of Life and Environmental Sciences, Prefectural University of Hiroshima, 562 Nanatsuka-cho, Shobara, Hiroshima 727-0023, Japan

^b Faculty of Health and Welfare Science, Okayama Prefectural University, 111 Kuboki, Soja, Okayama 719-1197, Japan

ARTICLE INFO

Article history:

Received 22 April 2016

Revised 18 May 2016

Accepted 10 June 2016

Available online 11 June 2016

Keywords:

Structure–activity relationships

RBL-2H3

Vanillic acid ester

Degranulation

ABSTRACT

Methyl vanillate (**1**) showed strong degranulation inhibitory activity among vanillin derivatives tested. In order to find structure–activity relationships for developing anti-allergic agents with simple structures and potent activity, we synthesized several vanillic acid (VA) ester derivatives with C₁–C₄ and C₈ alkyl chains and evaluated their degranulation inhibitory activities. The most active compound of VA ester derivatives was derivative **5** with a C₄ straight alkyl chain, and derivative **5** exhibited approximately three-fold greater inhibitory activity than that of **1**. Moreover, we designed 8 types of analogs based on **5**, and we found that the minimum structure for potent degranulation inhibitory activity requires direct connection of the butyl ester moiety on the benzene ring and at least one hydroxyl group on the benzene ring. Butyl *meta* or *para* hydroxyl benzoate (**10** or **11**) has a simpler structure than that of **5** and exhibited more potent degranulation inhibitory activity than that of **5**.

© 2016 Elsevier Ltd. All rights reserved.

Epidemiology has suggested that the prevalence of atopic dermatitis has increased over the past 30 years, and similar trends have been reported for asthma and hay fever.¹ These diseases are classified as immediate hypersensitivity (type I) allergy. The increasing prevalence of type I allergies has become a social problem, especially in developed countries. Type I allergy is caused by the cross-linking of high-affinity receptors for IgE (FcεRI) following IgE. The IgE–FcεRI complex with a specific antigen leads to the release of chemical mediators such as histamine, arachidonic acid metabolites, proteases, serotonin, cytokines and heparin.^{2–5} This phenomenon is called ‘degranulation’, and allergic reaction is caused by degranulation.

Recently, several drugs and natural products have been analyzed and have been shown to inhibit degranulation via suppression of signal pathways, suggesting that they could be valuable compounds for allergy therapy.^{6–12} However, the chemical structures are complex, and it is not clear how the structure affects degranulation inhibitory activity. Even if the active body is revealed, it would be difficult to produce anti-allergic drugs with a complex chemical structure on an industrial scale. The development of an anti-allergic agent that has a simple chemical structure and shows potent degranulation inhibitory activity is needed. We

focused on vanillic acid (VA) and its derivatives as compounds with simple chemical structures. These compounds naturally occur in plants. There have been several reports showing that they have multiple physiological functions including antioxidative activity,^{13,14} anti-inflammatory effect¹⁵ and restorative effect on a primary CoQ deficiency.¹⁶ As for immune functions, VA has been reported to have inhibitory activity against ulcerative colitis¹⁷ and hepatoprotective activity¹⁸ by suppressing immune mediators such as interleukin-6, interferon-γ and tumor necrosis factor-α. Although VA and its derivatives have been shown to have multiple physiological functions, there has been no report about their anti-allergy activity. In this study, we investigated chemical structures that have potent degranulation inhibitory activity by screening several derivatives and analogs based on the structure of VA.

Firstly, we evaluated anti-degranulation activity using vanillin (VN), VA and methyl vanillate (**1**), which are contained in many plants and are classified as phenolic compounds. Oxatomide, which is clinically used as an anti-allergy drug, was used as a positive control for the assay. As a result, compound **1** at a concentration of 1000 μM showed inhibitory activity against chemical mediator release from RBL-2H3, but VN and VA did not (Fig. 1). Ferulic acid, which is metabolized to vanillin and vanillic acid, had less anti-degranulation activity than did **1** (data not shown). These results suggested that the relatively lipophilic VA ester had more potent inhibitory degranulation activity than that of

* Corresponding author. Fax: +81 824 74 1779.

E-mail address: atai@pu-hiroshima.ac.jp (A. Tai).

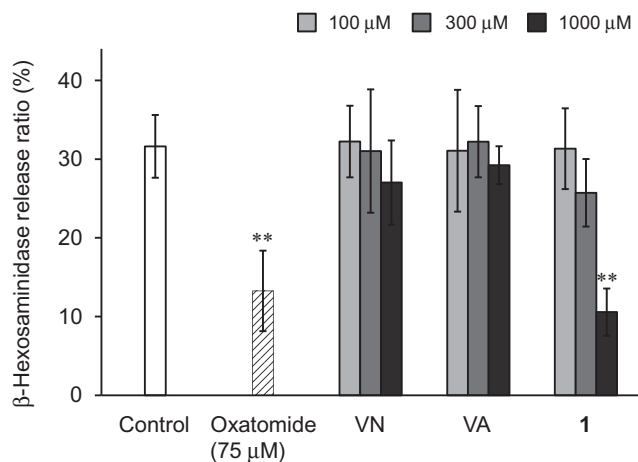


Figure 1. Inhibitory effects of VN, VA and **1** on antigen-induced degranulation in RBL-2H3 cells. DNP-IgE-sensitized RBL-2H3 cells were incubated with 75 μM oxatamide for a positive control or with indicated concentrations of these samples for 20 min and stimulated with DNP-HSA for 1 h. Release of β -hexosaminidase was measured. Each value is the mean of three independent cultures, and the bars show S.D. ** $p < 0.01$ (Dunnett's test) as compared with the control.

hydrophilic VN and VA in the anti-degranulation assay. It has been reported that membrane permeability and metabolic rate vary depending on the type (straight or branched) or length of lipophilic chains.^{19–21} Hence, degranulation inhibitory activity of a series of VA esters possessing ester chains of varying lengths from C_1 to C_4 and C_8 was investigated (Fig. 2A). VA ester derivatives **3**, **4** and **6** were obtained by coupling VA and the corresponding alcohol by using acetyl chloride as an acid catalyst (see Supplementary data). The structures of all of the compounds were confirmed by spectroscopies (^1H and ^{13}C NMR and mass spectrometry, see Supplementary data). With increasing ester chain length, VA esters generally tended to have poor solubility in the culture medium. To solubilize the compounds, α -cyclodextrin (α -CD) was used in this assay. α -CD has been reported to have an 'inclusion effect', and this effect increases the solubility of guest molecules.²² All of the compounds were dissolved in a culture medium containing α -CD (final concentration of 300 μM). α -CD at a concentration of 300 μM has been shown not to affect the activity of VA esters in this assay.

The degranulation inhibitory activity of VA ester derivatives possessing straight chains from C_1 to C_4 tended to increase with increasing length of their ester group (Fig. 2B). Derivative **5** with a C_4 straight chain ($\text{IC}_{50} = 198 \mu\text{M}$) showed approximately three-fold higher inhibitory activity than that of original compound **1** with a C_1 chain ($\text{IC}_{50} = 640 \mu\text{M}$). In contrast, a branched-chain ester had lower activity than that of a straight-chain ester. Actually, the potency of derivative **4** with a C_3 branched chain ($\text{IC}_{50} = 415 \mu\text{M}$) was approximately 30% lower than that of derivative **3** possessing a C_3 straight chain ($\text{IC}_{50} = 294 \mu\text{M}$). The VA ester with the longest chain, derivative **6**, increased degranulation caused by cytotoxicity (data not shown). Thus, the results of this assay revealed that derivative **5** had suitable lipophilicity, water solubility and potent degranulation inhibitory activity without cytotoxicity. It is possible that VA ester derivatives taken into the cells were cleaved by the cell surface esterase or intracellular esterase and the resulting metabolite mixture exhibited inhibitory activity. However, metabolites of **5**, VA and butanol did not show an inhibitory effect (data not shown). Considering these data, it seems that VA esters showed activity at the cell surface and/or in the intracellular compartment before hydrolysis of VA esters by esterase and that the lipophilic side chain of VA esters played an important role in the membrane permeability and distribution of VA esters in mast cells.

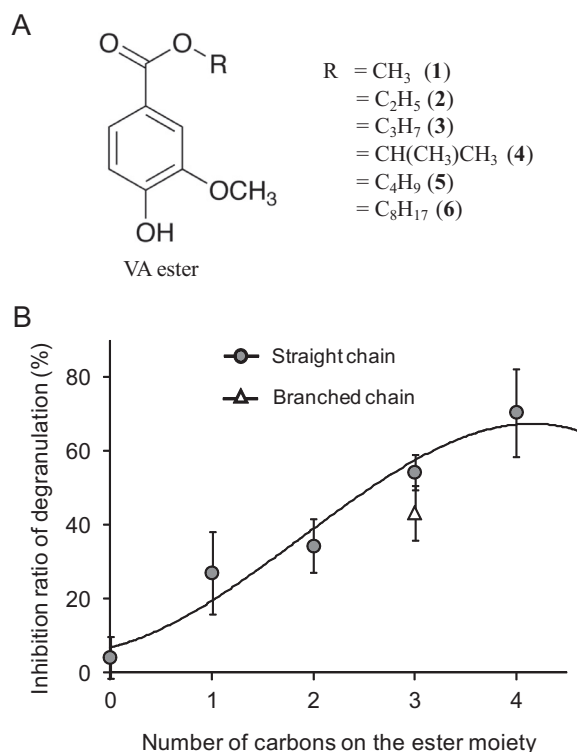


Figure 2. Structures and degranulation inhibitory activity of VA esters. (A) Structures of VA esters. (B) Influence of the ester moiety of VA ester on degranulation inhibitory activity. The data show the inhibition ratio of degranulation in each ester group at 300 μM . Each value is the mean of three independent cultures, and the bars show SD.

Table 1
Structures of synthesized compounds for designs 1 and 2

Compound	Design			
	1	2	3	4
	R ¹	R ²	R ³	R ⁴
7	C ₃ H ₇	CH ₂	OH	OCH ₃
8	C ₂ H ₅	C ₂ H ₄	OH	OCH ₃
9	C ₄ H ₉	—	OCH ₃	OH
10	C ₄ H ₉	—	OH	H
11	C ₄ H ₉	—	H	OH
12	C ₄ H ₉	—	OCH ₃	H
13	C ₄ H ₉	—	H	OCH ₃
14	C ₄ H ₉	—	H	H

The results indicated that derivative **5** per se with a C_4 straight-chain ester exhibited degranulation inhibitory activity.

VA esters have a hydroxyl group at the *para* position and a methoxy group at the *meta* position on the benzene ring and are compounds having an ester group as the lipophilic moiety. It was investigated whether the position of the oxycarbonyl group in the lipophilic chain was important for exhibiting degranulation

Download English Version:

<https://daneshyari.com/en/article/1368508>

Download Persian Version:

<https://daneshyari.com/article/1368508>

[Daneshyari.com](https://daneshyari.com)