Toxicology Reports 3 (2016) 701–707

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Trimellitic anhydride induces low-grade mast cell degranulation without specific IgE

Kazuhiro Ogi (M.D., Ph.D)*, Tetsuji Takabayashi, Takechiyo Yamada, Masafumi Sakashita, Masafumi Kanno, Norihiko Narita, Shigeharu Fujieda

Division of Otorhinolaryngology Head and Neck Surgery, Department of Sensory and Locomotor Medicine, Faculty of Medical Sciences, University of Fukui, Fukui 910-1193, Japan

ARTICLE INFO

Article history:
Received 14 March 2016
Received in revised form 1 September 2016
Accepted 2 September 2016
Available online 7 September 2016

Keywords:
Occupational rhinitis
Respiratory hypersensitivity
Trimellitic anhydride
Mast cell

ABSTRACT

Objectives: Low-molecular-weight (LMW) substances are known to be causative agents of occupational asthma (OA) and occupational rhinitis (OR). Although most LMW substances are irritants or allergens, some can cause immediate type immunoglobulin E (IgE)-mediated allergic reactions. Trimellitic anhydride (TMA) is one such LMW substance, which is known as an immunological sensitizer. However, the exact molecular biological details of the effects of TMA remain unclear.
Methods: We measured the β-hexosaminidase release from mast cells after directly exposing the cells to various LMW substances. The tyrosine phosphorylation of whole cellular molecules and the phosphorylation of extracellular signal-regulated kinase (ERK) were assessed by immunoblot assay.
Results: Among the LMW substances tested, only TMA induced β-hexosaminidase release. However, the mast cell degranulation induced by TMA was lower than that induced by an antigen or a calcium ionophore. Moreover, the pattern of tyrosine phosphorylation of whole cellular molecules was quite different between IgE-mediated antigen stimulation and TMA exposure. The TMA effect on mast cells was independent of not only IgE but also Ca^{2+} influx. ERK phosphorylation was not detected in mast cells exposed to TMA.
Conclusions: TMA induced mild degranulation of mast cells without IgE, even though the phosphorylation of ERK was not detected. This reaction suggests that TMA affects humans even upon first exposure. Therefore, it is imperative to avoid human exposure to high concentrations of TMA. In order to stop the development of severe asthma in individuals with OR, we need to be able to identify cases of OR caused by TMA as soon as possible.
© 2016 The Authors. Published by Elsevier Ireland Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Occupational exposure is regarded as one of the environmental factors responsible for allergic rhinitis. Occupational rhinitis (OR), defined as an inflammatory disease of the nasal mucous membrane without infection, develops in the work place. However, the disease is distinguished from work-exacerbated rhinitis, which is natural rhinitis aggravated by occupational exposure. Despite that OR is not a rare disease, its accurate diagnosis is difficult in some cases. It is very important to identify OR patients and to treat them appropriately in daily clinical cases. The major symptoms of OR are sneezing, rhinorrhea, and persistent or intermittent nasal obstruction. The cause includes substances that are significantly associated with the work place or occupational exposure, resulting in the decrease of nasal breath flow and excessive nasal secretions or rhinorrhea. There are two general classifications of OR [1,2], with the classification by Castano (i.e., immunological type and irritant-induced) currently being the main one. The immunological type is dependent on immunoglobulin E (IgE), and thus the mechanism of immune reaction is similar to that of normal allergic rhinitis. The causative agents of OR are divided into low-molecular-weight (LMW) and high-molecular-weight (HMW) substances [3]. Most HMW substances are glycoproteins of animal or plant origins. When humans are sensitized by HMW substances, an immediate IgE-mediated

Abbreviations: LMW, low molecular weight; OA, occupational asthma; OR, occupational rhinitis; IgE, immediate immunoglobulin E; TMA, trimellitic anhydride; HMW, high molecular weight; IgE, immunoglobulin E; IL, interleukin; DNP, dinitrophenylated; BSA, bovine serum albumin; PD, piecemeal degranulation.

* Corresponding author at: Division of Otorhinolaryngology Head and Neck Surgery, Department of Sensory and Locomotor Medicine, Faculty of Medical Science, University of Fukui, 23-3, Shimoaizuki, Matsuoka Eiheiji-cho Yoshida-gun, Fukui 910-1193, Japan.
E-mail address: ogikazu@u-fukui.ac.jp (K. Ogi).

http://dx.doi.org/10.1016/j.toxrep.2016.09.001
2214-7500/© 2016 The Authors. Published by Elsevier Ireland Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

allergic reaction ensues, resulting in OR. Similarly, some LMW substances can cause immediate IgE-mediated allergic reactions, but most work as irritants. It is postulated that irritants have either an irritation effect to cause neurogenic inflammation or a corrosive effect to inflict direct damage to airway epithelial cells; however, details of the exact mechanism remain unclear.

Mast cells play important roles in the development of allergic disease and the inflammatory process and in protection against gram-negative bacteria. A previous study using an animal model revealed that interleukin (IL)-33 and FcεRI play essential roles in the development of rhinitis [4]. The RBL-2H3 cell line is a mast cell line that was cloned, by the limited dilution technique, from leukemia cells isolated from rats that had been treated with a chemical carcinogen. RBL-2H3 cells have been used for studying IgE–FcεRI interactions [5] and signaling pathways for degranulation [6], as well as to test novel mast cell stabilizers [7].

The activation of mast cells is known to induce eosinophilic inflammation in the tissue. In this study, we sought to investigate the mechanism by which mast cells are affected after direct exposure to LMW substances. To this end, the β-hexosaminidase release assay was used to test several LMW substances. Upon the finding that trimellitic anhydride (TMA) was the only LMW substance that could cause β-hexosaminidase release, we studied this substance in further detail. By excluding Ca^{2+} from the buffer, we determined whether Ca^{2+} mobilization was essential for the TMA-induced phenomenon. In addition, we investigated the effect of TMA on mast cell signal transduction by immunoblot analysis.

2. Material and methods

Cell culture and low-molecular-weight substances. RBL-2H3 cells were maintained in Dulbecco's modified Eagle's medium containing 100 U/mL of penicillin and 10% heat-inactivated fetal calf serum. Diphenylmethane diisocyanate, toluene diisocyanate, hexamethylene diisocyanate, pyromellitic dianhydride, and TMA (Tokyo Chemical Industry, Tokyo, Japan) were used as commercial preparations dissolved in ethanol. Lithium chloride, cadmium chloride, nickel chloride hexahydrate, chromic chloride hexahydrate, cobalt chloride hexahydrate (Nacalai, Kyoto, Japan), ammonium persulfate, and ammonium thioglycolate (Sigma, St. Lois, MO, USA) were dissolved in water. These were each adjusted to 5 or 10 mM before adding to the medium with cells.

Analysis of β-hexosaminidase release. Antigen-induced degranulation was determined by the measurement of β-hexosaminidase release. RBL-2H3 cells (10^5) were seeded in 24-well plates and cultured overnight with anti-2,4-dinitrophenylated IgE mAb (anti-DNP IgE, clone SPE-7; Sigma). The cells were washed once with Tyrode-Hepes buffer (10 mM Hepes, pH 7.4, 127 mM NaCl, 4 mM KCl, 0.5 mM KH_2PO_4, 1 mM $CaCl_2$, 0.6 mM $MgCl_2$, 10 mM $LiCl_2$, 5.6 mM glucose, and 0.1% bovine serum albumin (BSA)) and then stimulated with 1–1000 ng/mL of the antigen DNP-BSA (LSL, Tokyo, Japan). The cells were also stimulated with 0.25–2 μM of the Ca^{2+} ionophore A23187 (Sigma) or 5–10 mM of LMW substances in the absence of anti-DNP IgE in the same buffer. After incubation for 1 h at 37 °C, the medium was recovered and reacted with the substrate p-nitrophenyl-N-acetyl-β-D-glucopyranoside (Nacalai) in 0.1 M sodium citrate buffer (pH 4.5) for 30 min at 37 °C. The reaction was terminated by the addition of 0.2 M glycine buffer (pH 10.7). The release of the product 4-P-nitrophenol was monitored by measuring its absorbance at 405 nm, using a microplate reader (Spectra Max 250; Molecular Devices, Sunnyvale, CA, USA). The released β-hexosaminidase activities were expressed as a percentage of maximal release induced by 1% NP-40 in Tyrode-HEPES buffer.

Table 1
Known causative low-molecular-weight substances of occupational rhinitis used in this study. A), B), C) are used in polyurethane resin factories. D), E) are used in epoxy resin factories. F), G), H), I), J) are metals used in electrical device factories. K), L) are used as hair dye.

A) Diphenylmethane diisocyanate	G) Cadmium chloride (II)
B) Toluene diisocyanete	H) Nickel chloride hexahydrate (II)
C) Hexamethylene diisocyanate	I) Chromic chloride hexahydrate (III)
D) Pyromellitic dianhydride	J) Cobalt chloride hexahydrate (II)
E) Trimellitic anhydride	K) Ammonium persulphate
F) Lithium chloride (II)	L) Ammonium thioglycolate

Immunoblotting. RBL-2H3 cells were seeded into a dish and cultured overnight with anti-DNP IgE mAb. The cells were washed twice with Tyrode-Hepes buffer and activated by 30 ng/mL of the DNP-BSA antigen or 10 mM of TMA. Cells were washed twice with ice-cold PBS and then solubilized in lysis buffer containing 1% NP40 (50 mM Tris, pH 7.4, 150 mM NaCl, 10 mM ethylenediaminetetraacetic acid, 100 mM NaF, 1 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride, 2 μg/mL aprotinin) on ice. Cell lysates were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and then electronically transferred to a polyvinylidene difluoride transfer membrane (Merck Millipore, Billerica, MA, USA). After blocking with 5% milk in TBST (25 mM Tris, pH 8.0, 150 mM NaCl, and 0.1% Tween 20), the blots on the membrane were reacted with the indicated primary antibodies, followed by horseradish peroxidase-conjugated second antibodies (Bio-Rad, Hercules, CA, USA) in TBST. Proteins were visualized by the enhanced chemiluminescence reagent (Western Lightning, PerkinElmer, Winter Street Waltham, MA, USA). Anti-phospho-extracellular signal-regulated kinase (ERK) (Thr202/Tyr402) and anti-ERK antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA).

Cell viability assays. RBL-2H3 cells were trypsinized after 1 h exposure to TMA or vehicle (ethanol), counted by trypan blue dye exclusion assay. The percentage of dead cells in living cells was shown as a result. All experiments were conducted independently in triplicate.

3. Results

Exposure to TMA induced mast cell degranulation. At first, we examined the effects of several LMW substances on mast cells. The substances we used in this study, all known causative agents of OR, are shown in Table 1. The β-hexosaminidase release was not observed with the same volume of vehicle (ethanol) that was used as controls (Fig. 1). Although the degranulation was induced in DNP-sensitized RBL-2H3 cells by DNP-BSA stimulation, we didn't find the effect of DNP-BSA in the absence of anti-DNP IgE on mast cells degranulation. Moreover, we also didn't find the effect of anti-DNP IgE in the absence of DNP-BSA on degranulation. The suspension of hexamethylene diisocyanate (C) was too turbid to allow measurement of β-hexosaminidase release. We found that among the LMW substances tested, only TMA induced β-hexosaminidase release. The β-hexosaminidase release was more obvious with 10 mM TMA than with the 5 mM concentration. This effect became smaller when 20 mM was used, and the phenomenon was not evident at 100 mM. We concluded that the effect of TMA was not dose dependent and used 10 mM as the optimal concentration in the subsequent experiments. On the other hand, pyromellitic dianhydride could not induce degranulation of mast cells despite that it is also an anhydride like TMA. However, the cells stimulated by TMA could release β-hexosaminidase without IgE. This suggested that the TMA-induced degranulation of mast cells is independent of IgE crosslinking. However, the degranulation induced by TMA was lower than that induced by the antigen or calcium ionophore.

Download English Version:

https://daneshyari.com/en/article/2572181

Download Persian Version:

https://daneshyari.com/article/2572181

Daneshyari.com