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Comparison of inhibitory activities of zinc oxide ultrafine and fine particulates on IgE-induced mast cell activation

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Abstract The effects of ultrafine and fine particles of zinc oxide (ZnO) on IgE-dependent mast cell activation were investigated. The rat mast cell line RBL2H3 sensitized with monoclonal anti-ovalbumin (OVA) IgE was challenged with OVA in the presence or absence of ZnO particles and zinc sulfate ($ZnSO_4$). Degranulation of RBL2H3 was examined by the release of β-hexosaminidase. To understand the mechanisms responsible for regulating mast cell functions, the effects of ZnO particles on the levels of intracellular Zn^{2+}, Ca^{2+}, phosphorylated-Akt, and global tyrosine phosphorylation were also measured. IgE-induced release of β-hexosaminidase was obviously attenuated by ultrafine ZnO particles and $ZnSO_4$, whereas it was very weakly inhibited by fine ZnO particles. The intracellular Zn^{2+} concentration was higher in the cells incubated with ultrafine ZnO particles than in those with fine ZnO particles. Consistent with inhibitory effect on release of β-hexosaminidase, ultrafine ZnO particles and $ZnSO_4$, but not fine ZnO particle, strongly attenuated the IgE-mediated increase of phosphorylated-Akt and tyrosine phosphorylations of 100 and 70 kDa proteins in RBL2H3 cells. These findings indicate that ultrafine ZnO particles, with a small diameter and a large total surface area/mass, could release Zn^{2+} easily and increase intracellular Zn^{2+} concentration efficiently, thus decreasing FcεRI-mediated mast cell degranulation through inhibitions of PI3K and protein tyrosine kinase activation. Exposure to ZnO particles might affect immune responses, especially in allergic diseases.

Keywords IgE · Nanoparticles · Degranulation · Mast cell · Zinc oxide · PI3K

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Introduction

Antigen stimulation induces an aggregation of IgE receptors (FcεRI) on the surface of IgE-sensitized mast cells and basophils, followed by releases of various mediators such as histamine, cysteinyl-leukotriens and enzymes including β-hexosaminidase. These mediators play a role in the appearance of major symptoms of type I allergic diseases such as vasodilation, mucous secretion, itching, and bronchoconstriction in allergic rhinitis, atopic dermatitis and asthma. Considering that the activation of mast cells and basophils associated with the diseases are modulated by many chemical compounds such as environmental factors (Devouassoux et al. 2002), nutrients (Zingg 2007), and drugs (Shin et al. 2004), concern over the effect of new compounds including

ultrafine particles of nanometer scale made by improved techniques on mast cell function and type I allergic responses has been growing.

Zinc oxide (ZnO) is regarded as a safe chemical and is used for cosmetics, drugs, and paints. The toxicological evaluation of ZnO has shown that the LD_{50} for rats is more than 5 g/kg body weight and the compound is non-toxic by single oral ingestion, and several studies have suggested that excess Zn^{2+} consumption through diet (Scientific Committee on Cosmetic Products and Non-food Products 2003) does not have any adverse effects in humans. However, ultrafine ZnO has recently become utilized as substitute for fine ZnO in industry. Because of its small size, ultrafine ZnO may be more easily inhaled and absorbed by humans via the airway, and occasionally the digestive organs and the skin. Moreover, there are a number of studies reporting that ZnO is biologically active. For instance, ZnO apparently enhanced the cellular uptake of anticancer drugs for a leukemia cell line (Guo et al. 2008) and is toxic to both gram-negative and gram-positive bacteria systems (Reddy et al. 2007). Therefore, for safe use, the potential effects of ZnO on various functions responsible for human health need to be clarified. Especially in the area of immunology, there have been few studies demonstrating the effects of ZnO on allergic responses. Moreover, the effect of ZnO on mast cell functions has not been investigated. Although some researchers reported that Zn^{2+} inhibits IgE-mediated degranulation of the mast cell line RBL2H3 (Hide and Beaven 1991), the mechanism by which it does so remains to be fully elucidated.

In the present study, to clarify whether ZnO modulates mast cell activation by FcεRI crosslinking, we tested the effects of ZnO on IgE-dependent degranulation, Zn^{2+} and Ca^{2+} mobilization, and the level of phosphorylated-Akt and global tyrosine phosphorylation of RBL2H3 cells. Additionally, experiments utilizing ultrafine ZnO and fine ZnO revealed that the effect of ZnO on mast cell activation is size-dependent.

Materials and methods

Cells

RBL2H3 cells were supplied by the Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan. Anti-ovalbumin (OVA) IgE producing hybridoma (OE-1 hybridoma) was established by using Polyethylene Glycol 1500 (Roche Diagnostics GmbH, Mannheim, Germany) following the manufacturer's protocol.

Degranulation of RBL2H3 cells

RBL2H3 cells were maintained in 5% CO_2 at 37°C in minimum essential medium (Invitrogen Japan K.K., Tokyo, Japan) containing 10% (v/v) fetal bovine serum (Invitrogen Japan K.K.), 100 units of penicillin and 100 units of streptomycin (Invitrogen Japan K.K.). The cells were harvested by trypsin (0.25%, w/v) (Invitrogen Japan K.K.) and EDTA (Wako Pure Chemical Industries, Ltd, Osaka, Japan) (0.01%, w/v) in phosphate-buffered saline and suspended at 0.25×10^6 cells/ml in medium containing 50 ng/ml of anti-OVA IgE mAb (OE-1) produced by OE-1 hybridoma. Five hundred microliters of the cell suspension was added to each well of 24-well cluster dishes and cultured for 24 h. The cells were washed with PIPES buffer (25 mM PIPES (pH 7.2), 119 mM NaCl, 5 mM KCl, 5.6 mM glucose, 0.4 mM $MgCl_2$, 1 mM $CaCl_2$ and 0.1% (w/v) bovine serum albumin) (Hirasawa et al. 1995) and stimulated for 20 min with OVA (Sigma–Aldrich Fine Chemicals, St. Louis, MI, USA) at a concentration of 5 μg/ml with the indicated concentrations of ultrafine ZnO (21 nm diameter) (Ishihara Sangyo Kaisha Inc., Osaka, Japan), fine ZnO (<5 μm diameter) (Wako Pure Chemical Industries, Ltd), or $ZnSO_4$ (Wako Pure Chemical Industries, Ltd). β-hexosaminidase in the cultured supernatant was determined by the colorimetric assay using *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide (Sigma–Aldrich Fine Chemicals). Values for β-hexosaminidase released in the medium were expressed as the percentages of the total β-hexosaminidase, which was determined in the cells lysed in 0.1% Triton X-100. The zinc compounds at the concentrations used did not affect the colorimetric assay.

Cytotoxicity assay for ZnO-treated RBL2H3 cells using MTT tetrazolium salt

After treatment of RBL2H3 cells with the zinc compounds and OVA, the cells were washed with the

minimum essential medium containing 10% (v/v) fetal bovine serum, 100 units of penicillin and 100 units of streptomycin prewarmed. The cells were further incubated for 4 h with the medium containing MTT (Sigma–Aldrich Fine Chemicals). After removal of the medium, the resultant colored products were dissolved in DMSO, and the absorbance at 562 nm was determined (Mosmann 1983).

Measurement of the levels of intracellular Zn^{2+} and Ca^{2+}

Measurements of the levels of intracellular Zn^{2+} and Ca^{2+} were performed using the zinc-and calcium-reactive fluorescence probes, FluoZin-3 (Gee et al. 2002) and Fluo-3 (Kao et al. 1989), respectively. Briefly, RBL2H3 cell suspensions (6×10^5 cells/ml in PIPES buffer) were incubated with the minimum essential medium containing 10% (v/v) fetal bovine serum, 100 units of penicillin, 100 units of streptomycin, anti-OVA IgE, and fluorescence probe (5 μM FluoZin-3-AM (Invitrogen Japan K.K.) with 0.02% Pluronic F127 (Invitrogen Japan K.K.) for Zn^{2+} measurement or 4 μM Fluo-3-AM (Dojindo Laboratories, Kumamoto, Japan) for Ca^{2+} measurement) for 30 min at 37°C and then washed twice with and resuspended in the PIPES buffer. The cells were then stimulated with OVA and the zinc compounds at 0 min and monitored for fluorescence (FL1) until 140 s by FACScaliver (BD Bioscience, San Jose, CA, USA).

Immunoblotting

After the stimulation, the cells in a 60 mm dish were lysed in 0.18 ml of ice-cold lysis buffer (20 mM PIPES, pH 8.0, 1% (v/v) Triton X-100, 1 mM EDTA, 50 mM NaF, 2.5 mM *p*-nitrophenyl phosphate, 1 mM Na_3VO_4, 0.02 mg/ml leupeptin and 10% (v/v) glycerol). The proteins in the cell lysate were separated by SDS–PAGE and transferred onto a PVDF membrane (ATTO, Tokyo, Japan). The phosphorylated-Akt and total-Akt were detected by immunoblotting using polyclonal antibodies for phospho-Akt (Ser473) and Akt (Cell Signaling Technology, Beverly, MA, USA), respectively. The level of global tyrosine phosphorylation was detected by using monoclonal antibodies for phosphorylated tyrosine (BioLegend, San Diego, CA, USA).

Statistics

To analyze data statistically, the ANOVA (Tukey) test was used for multiple comparisons.

Results

Ultrafine ZnO has a greater inhibitory activity on anti-OVA-specific IgE-dependent degranulation of RBL2H3 cells as compared to fine ZnO

In order to clarify whether ZnO modulates mast cell activation, we tested the effect of ultrafine ZnO and fine ZnO on IgE-mediated degranulation response of RBL2H3 cells. Even though ZnO is nearly insoluble in water, ultrafine ZnO strongly decreased FcεRI-mediated *β*-hexosaminidase release in a dose-dependent manner (Fig. 1a). Significant inhibition of FcεRI-mediated degranulation response was observed when the cells were treated by the compound at 5 and 10 μg/ml. On the other hand, fine ZnO significantly, but only weakly, inhibited the degranulation response at 10 μg/ml. Water soluble zinc compound, $ZnSO_4$, significantly and strongly inhibited the FcεRI-mediated degranulation (Fig. 1b) at 10 and 20 μg/ml, as did ultrafine ZnO. Roughly the same amount of zinc is contained in 20 μg/ml $ZnSO_4$ and 10 μg/ml ZnO. The zinc compounds themselves failed to affect the release of *β*-hexosaminidase from RBL2H3 cells (data not shown).

The viability of RBL2H3 cells assayed using MTT was not affected by the treatment with the zinc compounds at the concentrations used, suggesting that inhibition of release of the granule components by the compound was not induced by their cytotoxicity (data not shown).

Effects of ZnO on the intracellular Zn^{2+} and Ca^{2+} in RBL2H3 cells

Since ZnO and $ZnSO_4$ similarly attenuated mast cell degranulation, Zn^{2+} might play an important role in inhibiting mast cell activation. Moreover, the action of Zn^{2+} as second messenger was recently revealed (Yamasaki et al. 2007). In an attempt to prove that ZnO modifies the intracellular Zn^{2+} concentration and thus inhibits mast cell activation, the intracellular Zn^{2+} level was measured after exposure to the zinc

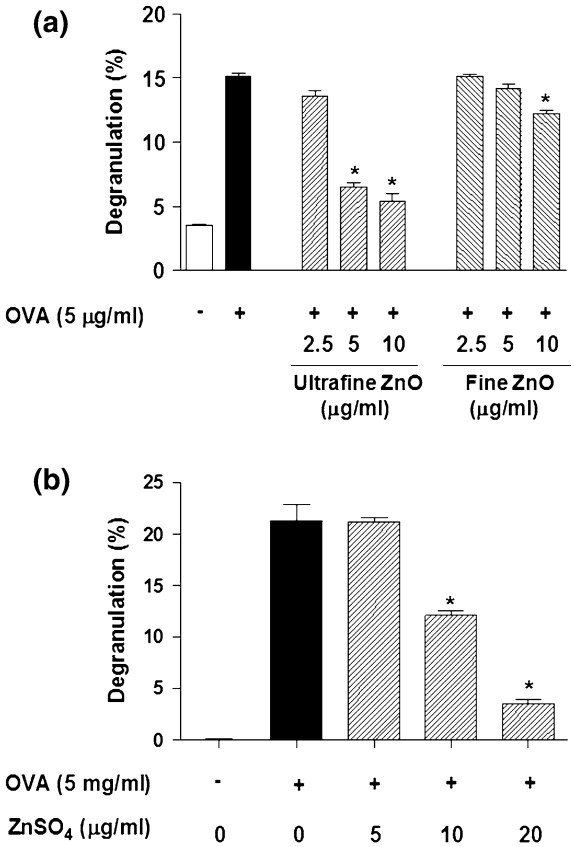


Fig. 1 Effect of ultrafine ZnO, fine ZnO (**a**), and $ZnSO_4$ (**b**) on the FcεRI-mediated release of β-hexosaminidase from RBL2H3 cells. RBL2H3 cells (2.5×10^5 cells/well) were incubated with anti-OVA IgE for 24 h. The cells were washed and then incubated with OVA (5 μg/ml) in the presence or absence of the zinc compounds. After 20 min, the releases of β-hexosaminidase from the cells were measured. * $P < 0.05$ versus absence of the compounds. *Bars* show mean + SEM of triplicated cultures of the cells. Data are representative of three experiments

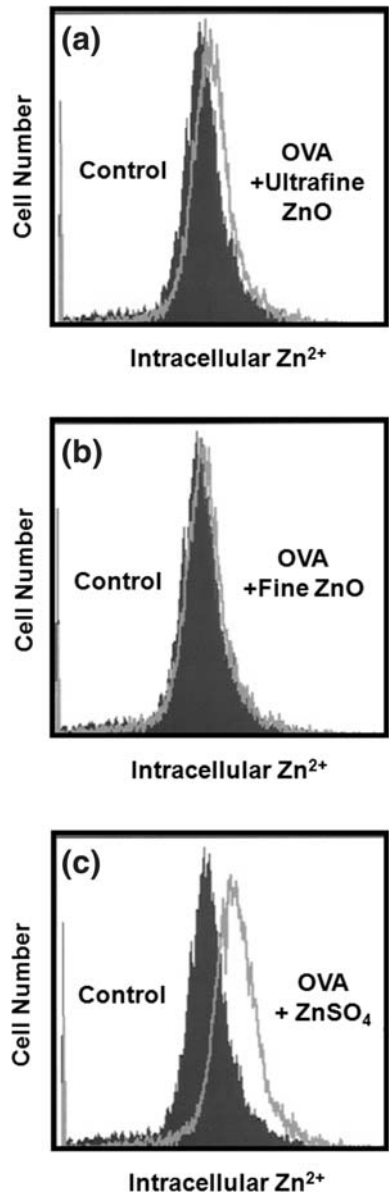


Fig. 2 Effect of ultrafine ZnO (**a**), fine ZnO (**b**), and $ZnSO_4$ (**c**) on the intracellular Zn^{2+} concentration in RBL2H3 cells. RBL2H3 cells (6×10^5 cells) were incubated with anti-OVA IgE and 5 μM FluoZin-3-AM at 37°C for 30 min. The cells were washed and stimulated for 5 min in the PIPES buffer containing OVA and the zinc compounds. Untreated and zinc compound-treated cells are indicated by *filled* and *open histograms*, respectively. Data are representative of three experiments

compounds. The level of intracellular Zn^{2+} concentration was greatly increased at 5 min after $ZnSO_4$ addition (Fig. 2). In parallel to a remarkable inhibitory effect on the degranulation, ultrafine ZnO obviously increased the intracellular Zn^{2+} level, whereas fine ZnO only scarcely increased it.

To address the question of how mast cell activation is inhibited by ZnO, the levels of intracellular Ca^{2+} in RBL2H3 cells were measured. Ninety-seconds after antigen stimulation, intracellular Ca^{2+} levels were increased in IgE-sensitized RBL2H3 cells (Fig. 3a). The stimulation-induced increase of the intracellular Ca^{2+} was not affected by the presence of ultrafine ZnO (Fig. 3b), fine ZnO (Fig. 3c), or $ZnSO_4$ (Fig. 3d). The zinc compounds themselves did not affect the intracellular Ca^{2+} concentration (data not

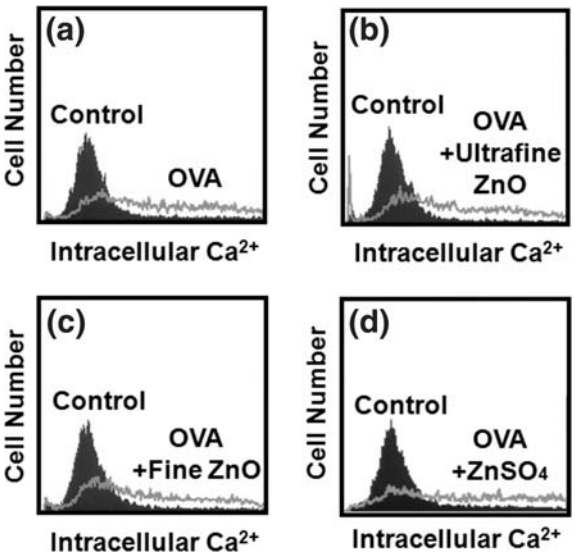


Fig. 3 Effect of ultrafine ZnO, fine ZnO, and $ZnSO_4$ on the FcεRI-mediated increase of intracellular Ca^{2+} concentration in RBL2H3 cells. RBL2H3 cells (6×10^5 cells) were incubated with anti-OVA IgE and 4 μM Fluo-3-AM at 37°C for 30 min. The cells were washed and stimulated for 90 s in the PIPES buffer containing OVA in the absence (**a**) or presence of the zinc compounds, ultrafine ZnO (**b**), fine ZnO (**c**), and $ZnSO_4$ (**d**). Unstimulated cells and cells stimulated by OVA with or without the zinc compound are indicated by *filled* and *open histograms*, respectively. Data are representative of three experiments

shown). The zinc compound also failed to affect the binding of anti-OVA IgE to the antigen in ELISA analysis (data not shown). These results indicated that the zinc compounds did not disturb crosslinking of IgE by antigen and the stimulatory input from FcεRI.

Effects of ZnO on the levels of phosphorylated-Akt and global tyrosine phosphorylation in RBL2H3 cells

Protein tyrosine kinases and PI3K are responsible for both FcεRI-mediated Ca^{2+}-dependent (Nishizumi and Yamamoto 1997; Ching et al. 2001) and -independent (Nishida et al. 2005; Gu et al. 2001) signaling pathways in mast cells. Thus, it is presumed that ZnO inhibits FcεRI-mediated Ca^{2+}-independent activation of mast cells via modulations of protein tyrosine kinase and PI3K activities. To further address the question of how the compounds inhibit mast cell activation, we tested the effects of ZnO on the levels of phosphorylated-Akt, as an indicator of PI3K activity, and global tyrosine phosphorylation. A certain level of phosphorylated-Akt was detected in non-stimulated RBL2H3 cells and slightly increased by ultrafine ZnO (Fig. 4a), fine ZnO (Fig. 4b) and $ZnSO_4$ (Fig. 4c) treatment. FcεRI-mediated activation by antigen increased the level of phosphorylated-Akt and the increase was inhibited by ultrafine ZnO (Fig. 4a) and $ZnSO_4$ (Fig. 4c), and to a lesser extent, fine ZnO (Fig. 4b).

Zinc compounds affected the tyrosine phosphorylation levels of some proteins in the antigen-stimulated and resting cells sensitized by anti-OVA IgE (Fig. 5). Most noteworthy, in accordance with its ability to lower Akt phosphorylation, ultrafine ZnO, as well as $ZnSO_4$, strongly lowered the obvious increases of tyrosine phosphorylation levels of two proteins (100 and 70 kDa, indicated by arrows in Fig. 5) by FcεRI crosslinking, whereas the tyrosine phosphorylation levels of these proteins in resting cells were increased by the compounds themselves. On the other hand, fine ZnO had little effect on the tyrosine phosphorylation levels of the two proteins in stimulated and resting cells.

Discussion

In this study, we demonstrated for the first time that ZnO diminished the release of β-hexosaminidase from the mast cell line RBL2H3 activated with anti-OVA IgE and its antigen via inhibitions of FcεRI-mediated PI3K and protein tyrosine kinase activations without affecting calcium mobilization. Our results also showed that ultrafine ZnO as well as $ZnSO_4$ inhibited FcεRI-mediated degranulation, suggesting that Zn^{2+} released from ZnO might be responsible for the inhibitory activity of ZnO. Furthermore, we firstly showed that ultrafine ZnO increased the intracellular Zn^{2+} concentration and inhibited mast cell activation more efficiently than fine ZnO.

Release of chemical mediators such as histamine and serotonin by degranulation of mast cells is an important step in type I allergic reactions and immune responses. Our results showed that ultrafine ZnO, and to a lesser extent, fine ZnO inhibited FcεRI-mediated degranulation of RBL2H3 cells (Fig. 1a), and thus this compound may influence immune and allergic reactions. Since another zinc compound, $ZnSO_4$, also

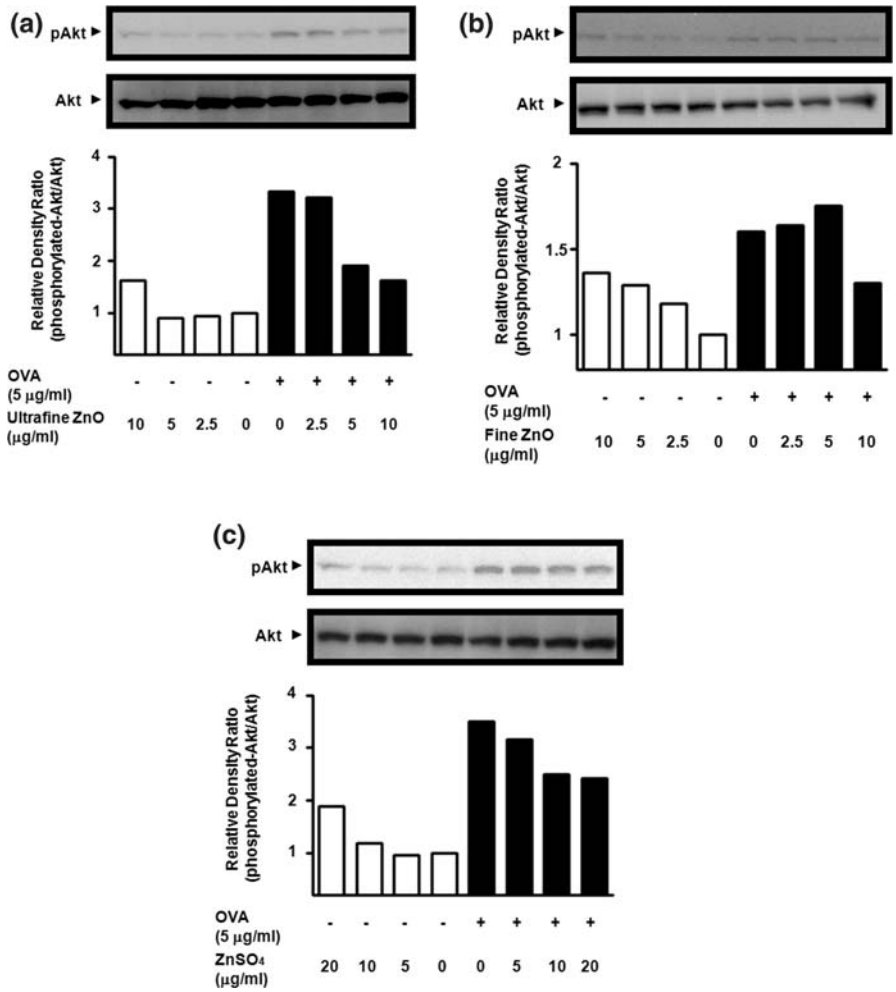


Fig. 4 Effect of ultrafine ZnO (**a**), fine ZnO (**b**), and $ZnSO_4$ (**c**) on the FcεRI-mediated phosphorylated-Akt level increase in RBL2H3 cells. RBL2H3 cells (7.5×10^5 cells) were sensitized by anti-OVA IgE for 24 h. The cells were washed and stimulated with OVA (5 μg/ml) in the presence or absence of ultrafine ZnO, fine ZnO, and $ZnSO_4$ for 10 min. Phosphorylated-Akt (pAkt) and Akt levels expressed in RBL2H3 were determined by Western blot. The *columns* in the graphs indicate the relative density ratio of the phosphorylated-Akt to the corresponding Akt as determined by densitometric analysis

inhibited FcεRI-mediated degranulation (Fig. 1b), it could be thought that Zn^{2+} released commonly from zinc compounds might inhibit the activation. This result was consistent with the previous reports that zinc chloride inhibits histamine release from human basophils (Marone et al. 1981). The inhibitory role of Zn^{2+} was also supported by the result that ultrafine ZnO and $ZnSO_4$, which strongly inhibited degranulation, obviously increased intracellular Zn^{2+} concentration (Fig. 2). Thus, Zn^{2+} released from ZnO was supposed to blunt the mast cell activation via modulation of the activities of intracellular signaling molecules.

The inhibition of degranulation of RBL2H3 cells by ZnO was not due to the attenuation of Ca^{2+}-dependent signaling pathways responsible for degranulation response, since the increased intracellular concentrations of Ca^{2+} by FcεRI-mediated activation were not inhibited by ZnO (Fig. 3a, b). $ZnSO_4$ also failed to affect Ca^{2+} mobilization induced by FcεRI crosslinking (Fig. 3c). In contrast to our result, Hide and Beaven (1991) reported that zinc attenuates IgE-induced degranulation via inhibition of calcium influx. The exact reason for this discrepancy is not known, but the most likely explanation is that free Zn^{2+} concentration in medium in our experiment might be lower than it in their experiment. Since ZnO is nearly insoluble in water, the free zinc amount utilized by cells in culture medium should be only a part of zinc added. Even zinc from the water soluble zinc compound, $ZnSO_4$, might form the insoluble zinc salt gradually, namely zinc hydroxide, in medium used at pH 7.2. Because of the lower Zn^{2+} concentration in the medium in the present study, it is considered that we could not observe the inhibitory effect of zinc compounds on Ca^{2+} mobilization.

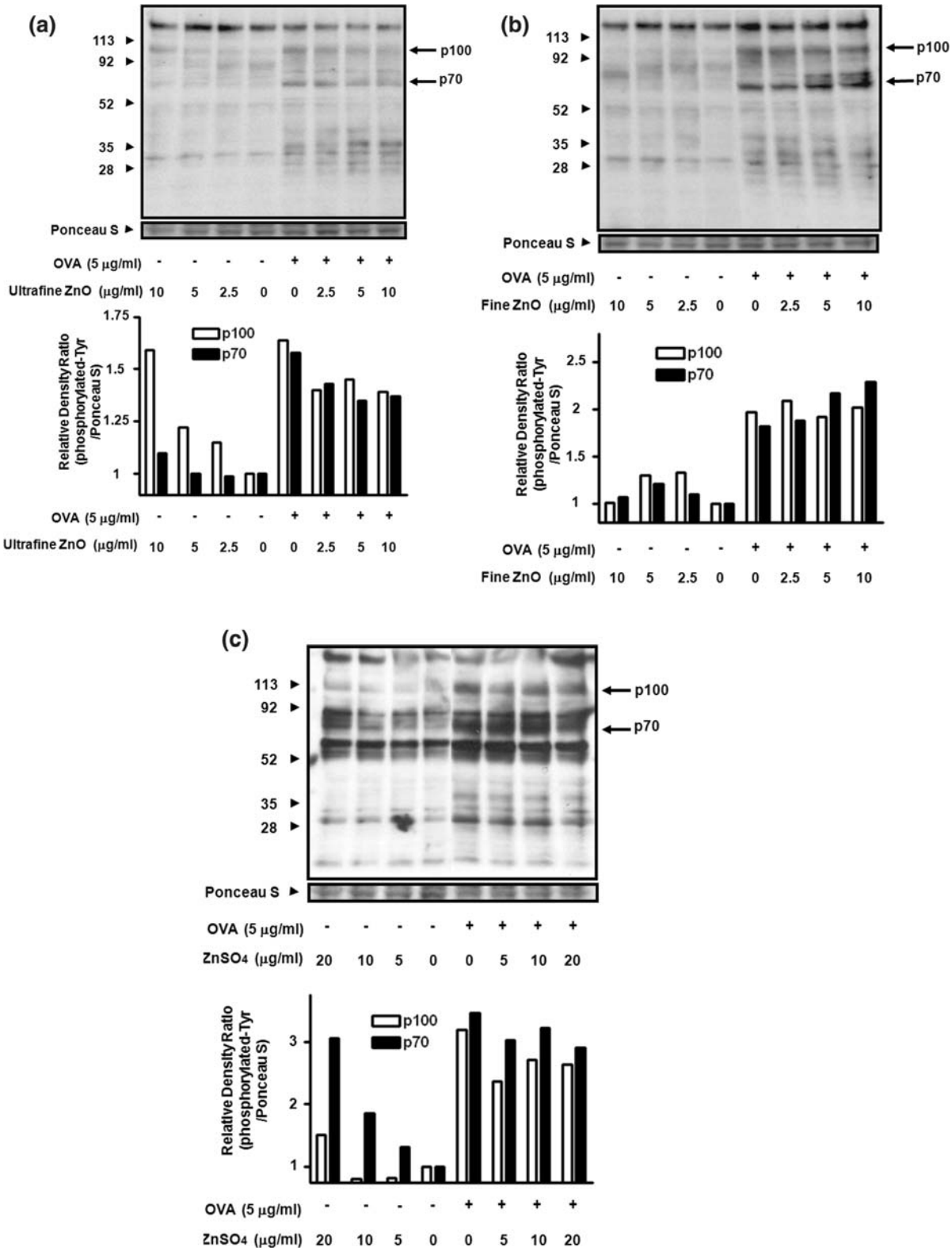
Interestingly, Beaven et al. (1984) mentioned that Zn^{2+} blocks histamine release (>95%) at concentrations where a significant Ca^{2+} signal is still observed by FcεRI-mediated RBL2H3 activation. Moreover, Musset et al. (2008) also described that Ca^{2+} influx stimulated by anti-IgE in human basophils was not inhibited by up to 300 μM Zn^{2+}, whereas 100 μM Zn^{2+} completely inhibited histamine release. Taken together, these results suggest that the inhibitory effect of zinc on Ca^{2+} mobilization, if any, is not critical for its inhibitory effect on degranulation.

On the other hand, ultrafine ZnO, as well as $ZnSO_4$, inhibited FcεRI-mediated PI3K activation (Fig. 4), which have a role in FcεRI-mediated Ca^{2+}-independent signaling pathway (Nishida et al. 2005). Thus, the possibility is considered that Zn^{2+} inhibits FcεRI-mediated degranulation via the inhibition of PI3K. However, many reports indicated that Zn^{2+} acts as a PI3K activator. For example, zinc chloride induced the phosphorylation of Akt (Ryu et al. 2009). The activation of the PI3K pathway following the intracellular rise of Zn^{2+} in cultured ZnT-1 expressed seminiferous tubule cells was also observed (Kaisman-Elbaz et al. 2009). Thus, the effect of Zn^{2+} on PI3K activity seems to vary with the cell condition. It is considerable that Zn^{2+} activates PI3K in the absence of other stimuli, whereas Zn^{2+} inhibits PI3K activation induced by other stimuli. Our result that the increase of Akt phosphorylation by Zn^{2+} treatment alone (Fig. 4) is also supported the assumption.

The zinc compounds changed tyrosine phosphorylation levels of some proteins (Fig. 5), which is modulated by various molecules including protein tyrosine kinases. A series of protein tyrosine kinases also participates in FcεRI-mediated Ca^{2+}-independent signaling pathway (Gu et al. 2001). Especially, ultrafine ZnO and $ZnSO_4$ commonly lowered the FcεRI-mediated increase of phosphorylation level of the band at about 70 kDa molecular weight in Fig. 5, which is suggested to be Syk by Moriya et al. (1997). Recently, Yu et al. (2006) reported that Syk is critical for FcεRI-evoked Gab2/PI3K activation. Together, these results suggested that Zn^{2+} might affect the critical upstream molecules of Syk and thus inhibit PI3K activation to attenuate FcεRI-mediated degranulation of RBL2H3 cells. In contrast, Parravicini et al. (2002) demonstrated that Fyn, not Syk, is required for FcεRI-mediated Gab2/PI3K activation. It is also possible that Zn^{2+} might affect the other tyrosine kinases such as Fyn and the subsequent PI3K-dependent pathway to decrease degranulation induced by IgE and antigen. Since the zinc compounds failed to inhibit morphological change of the cells induced by IgE and antigen stimulation (data not shown), Zn^{2+} probably impaired only one or several branched signaling pathways, namely Ca^{2+}-independent pathways. However, the effect of zinc on tyrosine kinase activation is controversial. In contrast to our results, compulsory increase of intracellular Zn^{2+} concentration by addition of $ZnSO_4$ and zinc ionophore pyrithione upregulated the increase of global tyrosine phosphorylation level induced by FcεRI-mediated stimulation via the inhibition of phosphatases (Yamasaki et al. 2007). Zinc depletion by the cell permeable zinc chelator TPEN inhibits mast cell degranulation without affecting the level of global tyrosine phosphorylation (Kabu et al. 2006). It was recently reported that zinc acts as an intracellular second messenger by induction of the zinc wave (Yamasaki et al. 2007). Therefore, strict modulation of intracellular zinc distribution might be quite important for protein tyrosine kinase activity in mast cells. Different effects among the various extracellular zinc sources on the zinc wave might be reason for the discrepancy between our results and other reports.

As shown in Fig. 2, a more profound increase of intracellular Zn^{2+} concentration in the presence of ultrafine ZnO, compared to fine ZnO, was observed. This result might indicate that smaller ZnO particles could release Zn^{2+} easily and increase the intracellular Zn^{2+} concentration efficiently, because of its large total surface area/mass. On the other hand, some reports indicated that ultrafine particles of other compounds also induced stronger biological responses than fine particles in vivo (Granum et al. 2001; Takafuji

Fig. 5 Effect of ultrafine ZnO (**a**), fine ZnO (**b**) and $ZnSO_4$ (**c**) on the FcεRI-mediated global tyrosine phosphorylation in RBL2H3 cells. RBL2H3 cells (7.5×10^5 cells) were sensitized by anti-OVA IgE for 24 h. The cells were washed and stimulated with OVA (5 μg/ml) in the presence or absence of ultrafine ZnO, fine ZnO, and $ZnSO_4$ for 5 min. Whole cell lysates were blotted with the anti-phosphotyrosine antibody PY20. *Arrows* indicated the proteins whose enhanced tyrosine phosphorylation by FcεRI-mediated stimulation was obviously attenuated by the compounds. A representative section of Ponceau S-stained membrane is shown as loading control. The *columns* in the graphs indicate the relative density ratios of p100 (*open column*) and p70 (*filled column*) to the corresponding ponceau S staining as determined by densitometric analysis


(a)
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Ultrafine ZnO (μg/ml) 10 5 2.5 0 0 2.5 5 10
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Relative Density Ratio (phosphorylated-Tyr /Ponceau S)
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Fine ZnO (μg/ml) 10 5 2.5 0 0 2.5 5 10
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OVA (5 μg/ml) - - - - + + + +
ZnSO4 (μg/ml) 20 10 5 0 0 5 10 20

et al. 1989; de Haar et al. 2006). Therefore, it is possible that the facilitated biological activities of ultrafine ZnO might be depends on both their rapid solubility and characteristics as nanoparticles.

Exposures of ZnO and the other oxidized metals to the welder causes metal fume fever, a flu-like syndrome (Gordon and Fine 1993). Serum zinc levels ranged from 0.55 to 1.5 μg/ml (equivalent to 0.68–1.87 μg/ml ZnO) in control subjects and were increased in patients suffered from metal fume fever after cutting galvanized steel in a poorly ventilated area for 6 h (Noel and Ruthman 1988). Therefore, the topical level of zinc in humans who are heavily exposed to zinc compounds, such as welders, might be comparable to the concentration used in our experiments (2.5–10 μg/ml ZnO).

Zinc compounds have long been used for treatment of a variety of diseases (Prasad 1979) and nutritional zinc supplementation is beneficial for prevention and treatment of human disease (Haase et al. 2008). In rodents, zinc markedly inhibits histamine releases from mast cells (Kazimierczak and Maslinski 1974). Our data demonstrate the inhibitory mechanisms of zinc compounds on mast cell activation in detail. Ultrafine ZnO, but not fine ZnO, efficiently inhibited FcεRI-mediated degranulation through attenuations of FcεRI-mediated PTK, and PI3K activation without affecting calcium mobilization of RBL2H3 cells in vitro via increasing intracellular Zn^{2+} concentration. Thus, a zinc compound such as ZnO is considered to modulate allergic diseases in humans such as allergic rhinitis and asthma by modifying mast cell functions.

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References

Beaven MA, Rogers J, Moore JR, Hesketh TR, Smith GA, Metcalfe JC (1984) The mechanism of calcium signal and correlation with histamine release in 2H3 cells. J Biol Chem 259:7129–7136

Ching TT, Hsu AL, Johnson AJ, Chen CS (2001) Phosphoinositide 3-kinase facilitates antigen-stimulated Ca^{2+} influx in RBL-2H3 mast cells via a phosphatidylinositol 3,4,5-triphosphate-sensitive Ca^{2+} entry mechanism. J Biol Chem 276:14814–14820

de Haar C, Hassing I, Bol M, Bleumink R, Pieters R (2006) Ultrafine but not fine particulate matter causes airway inflammation and allergic airway sensitization to co-administered antigen in mice. Clin Exp Allergy 36:1469–1479

Devouassoux G, Saxon A, Metcalfe DD, Prussin C, Colomb MG, Brambilla C, Diaz-Sanchez D (2002) Chemical constituents of diesel exhaust particles induce IL-4 production and histamine release by human basophils. J Allergy Clin Immunol 109:847–853

Gee KR, Zhou Z-L, Ton-That D, Sensi SL, Weiss JH (2002) Measuring zinc in living cells: a new generation of sensitive and selective fluorescent probes. Cell Calcium 31: 245–251

Gordon T, Fine JM (1993) Metal fume fever. Occup Med 8:504–517

Granum B, Gaarder PI, Groeng E, Leikvold R, Namork E, Lovik M (2001) Fine particles of widely different composition have an adjuvant effect on the production of allergen-specific antibodies. Toxicol Lett 118:171–181

Gu H, Saito K, Klaman LD, Shen J, Fleming T, Wang YP, Pratt JC, Lin G, Lim B, Kinet J-P, Neel BG (2001) Essential role for Gab2 in the allergic response. Nature 412:186–190

Guo D, Wu C, Jiang H, Li Q, Wang X, Chen B (2008) Synergistic cytotoxic effect of different sized ZnO nanoparticles and daunorubicin against leukemia cancer cells under UV irradiation. Photochem Photobiol B. 93:119–126

Haase H, Overbeck S, Rink L (2008) Zinc supplementation for the treatment or prevention of disease: current status and future perspectives. Exp Gerontol 43:394–408

Hide M, Beaven MA (1991) Calcium influx in a rat mast cell (RBL-2H3) line. J Biol Chem 266:15221–15229

Hirasawa N, Santini F, Beaven MA (1995) Activation of the mitogen-activated protein kinase/cytosolic phospholipase A_2 pathway in a rat mast cell line. J Immunol 154:5391–5402

Kabu K, Yamasaki S, Kamimura D, Ito Y, Hasegawa A, Sato E, Kitamura H, Nishida K, Hirano T (2006) Zinc is required for FcεRI-mediated mast cell activation. J Immunol 177: 1296–1305

Kaisman-Elbaz T, Sekler I, Fishman D, Karol N, Forberg M, Kahn N, Hershfinkel M, Silverman WF (2009) Cell death induced by zinc and cadmium is mediated by clusterin in cultured mouse seminiferous tubules. J Cell Physiol 220: 222–229

Kao JP, Harootunian AT, Tsien RY (1989) Photochemically generated cytosolic calcium pulses and their detection by fluo-3. J Biol Chem 264:8179–8184

Kazimierczak W, Maslinski C (1974) Histamine release from mast cells by compound 48/80. The membrane action of zinc. Agents Actions 4:320–323

Marone G, Findlay SR, Lichtenstein LM (1981) Modulation of histamine release from human basophils in vitro by physiological concentrations of zinc. J Pharmacol Exp Ther 217: 292–298

Moriya K, Rivera J, Odom S, Sakuma Y, Muramato K, Yoshiuchi T, Miyamoto M, Yamada K (1997) ER-27319, an acridone-related compound, inhibits release of antigen-induced allergic mediators from mast cells by selective inhibition of Fcε receptor I-mediated activation of Syk. Proc Natl Acad Sci 94:12539–12544

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63

Musset B, Morgan D, Cherny VV, MacGlashan DW Jr, Thomas LL, Rios E, DeCoursey TE (2008) A pH-stabilizing role of voltage-gated proton channels in IgE-mediated activation of human basophils. Proc Natl Acad Sci 105: 11020–11025

Nishida K, Yamasaki S, Ito Y, Kabu K, Hattori K, Tezuka T, Nishizumi H, Kitamura D, Goitsuka R, Geha RS, Yamamoto T, Yagi T, Hirano T (2005) FcεRI-mediated mast cell degranulation requires calcium-independent microtubule-dependent translocation of granules to the plasma membrane. J Cell Biol 170:115–126

Nishizumi H, Yamamoto T (1997) Impaired tyrosine phosphorylation and Ca^{2+} mobilization, but not degranulation, in lyn-deficient bone marrow-derived mast cells. J Immunol 158:2350–2355

Noel NE, Ruthman JC (1988) Elevated serum zinc levels in metal fume fever. Am J Emerg Med 6:609–610

Parravicini V, Gadina M, Kovarova M, Odom S, Gonzalez-Espinosa C, Furumoto Y, Saitoh S, Samelson LE, O'Shea JJ, Rivera J (2002) Fyn kinase initiates complementary signals required for IgE-dependent mast cell degranulation. Nat Immunol 3:741–748

Prasad AS (1979) Clinical, biochemical, and pharmacological role of zinc. Annu Rev Pharmacol Toxicol 19:393–426

Reddy KM, Feris K, Bell J, Wingett DG, Hanley C, Punnoose A (2007) Selective toxicity of zinc oxide nanoparticles to prokaryotic and eukaryotic systems. Appl Phys Lett 90: 2139021–2139023

Ryu JM, Lee MY, Yun SP, Han HJ (2009) Zinc chloride stimulates DNA synthesis of mouse embryonic stem cells: involvement of PI3K/Akt, MAPKs, and mTOR. J Cell Physiol 218:558–567

Scientific Committee on Cosmetic Products and Non-food Products (SCCNFP) (2003) Evaluation and opinion on: zinc oxide. 24th plenary meeting, Brussels

Shin HY, Kim JS, An NH, Park RK, Kim HM (2004) Effect of disodium cromoglycate on mast cell-mediated immediate-type allergic reactions. Life Sci 74:2877–2887

Takafuji S, Suzuki S, Koizumi K, Tadokoro K, Ohashi H, Muranaka M, Miyamoto T (1989) Enhancing effect of suspended particulate matter on the IgE antibody production in mice. Int Arch Allergy Appl Immunol 90:1–7

Yamasaki S, Sakata-Sogawa K, Hasegawa A, Suzuki T, Kabu K, Sato E, Kurosaki T, Yamashita S, Tokunaga M, Nishida K, Hirano T (2007) Zinc is a novel intracellular second messenger. J Cell Biol 177:637–645

Yu M, Lowell CA, Neel BG, Gu H (2006) Scaffolding adapter Grb2-associated binder 2 requires Syk to transmit signals from FcεRI. J Immunol 176:2421–2429

Zingg JM (2007) Vitamin E and mast cells. Vitam Horm 76: 393–418