A rapid and sensitive assay based on particle analysis for cell
degranulation detection in basophils and mast cells

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ABSTRACT

The degranulation of mast cells and basophils is often initiated by a number of pathophysiological responses, especially in allergic and inflammatory conditions. Efficient techniques and methods for determining the level of such degranulation are highly demanded for laboratory and clinical studies. In this work, a rapid and sensitive assay based on the particle analysis of granules in RBL-2H3 cells, a cell line widely used as a convenient model system to study the degranulation of mast cells and basophils, was developed to detect cell degranulation using a Nanosight NS300 in light scatter mode and dynamic light scattering (DLS) on a Malvern Zetasizer Nano-ZS instrument. Using this method, drug-induced mast cell degranulation and systemic anaphylaxis were efficiently determined both in cell culture medium and blood samples from animals in the current study. This promising method is expected to be widely used for screening anti-allergic and anti-inflammatory drugs both in vitro and in vivo models, as well as for determining the level of mast cell degranulation of the patients in the clinic.

1. Introduction

Basophils and mast cells are granulated hematopoietic cells that reside in nearly all tissues and the blood, and they are essential for many biological responses, such as allergic diseases and inflammatory disorders. They are characterized by their biphasic responses to stimuli, which involve the rapid degranulation (exocytosis particles) and immediate release of preformed inflammatory mediators (such as histamine and β-hexosaminidase) [1], which further induce the synthesis of arachidonic acid metabolites and the generation of cytokines and chemokines, finally leading to anaphylaxis [2].

Methods for the identification of mast cell degranulation have primarily used enzyme-linked immunosorbent assays (ELISAs) or colorimetric assays to measure preformed inflammatory mediators, such as histamine and β-hexosaminidase. The inflammatory mediators appear to be quickly released via exocytosis by the matrix of particles, called as granules that contain some bioactive mediators, and these mediators are regarded as the most important markers to detect the degranulation of mast cells. Histamine is a major mediator in allergic diseases and has multiple effects that are mediated by specific surface receptors on target cells. Thus, histamine release was developed to detect mast cell degranulation [3]. However, histamine has a half-life of approximately 1 min in the extracellular fluid and is degraded by histamine N-methyltransferase or diamine oxidase to tele-methylhistamine or imidazole acetaldehyde, respectively. Therefore, it is difficult to measure the level of histamine in extracellular fluids [4]. As another preformed inflammatory mediator in granules of mast cells, β-hexosaminidase functions as a potent inflammatory mediator stored in mast cells and is often released in parallel with histamine from activated mast cells. The determination of β-hexosaminidase is widely used to evaluate the level of mast cell degranulation [5]. Because β-hexosaminidase release is slow and the process persists for longer than histamine release does, β-hexosaminidase is highlighted as a better candidate for mast cell degranulation detection than histamine. Currently, the most widely used method for quantifying β-hexosaminidase is a colorimetric assay, which is composed of a number of steps that include cell sensitization with stimulated chemicals, the removal of colorless cell supernatants for the enzyme-substrate reaction, and spectrophotometric measure-
ments of 4-nitrophenol production. The enzyme-substrate reaction for this assay is typically carried out at a low pH (4.4–4.7), which is optimal for β-hexosaminidase activity, and is followed by an increase of the pH (9.8–11.7) to quench the enzymatic reaction. However, this method is largely limited to monitor enzyme activity because the complicated operational steps of β-hexosaminidase determination are inconvenient for rapid detection, high-throughput screening or real-time kinetic studies. Therefore, developing a more rapid, sensitive method to monitor mast cell degranulation is of vital importance for mast cell research.

In recent decades, nanotechnology has been used in the study of Chemistry, Physics, Materials, Biology and Medicine. Some novel methods that rely on nanotechnology have also been developed. For example, a self-assembled l-cysteine/gold nanoparticle was introduced into electrochemical biosensors to monitor IgE-mediated mast cell sensitization and activation, which implies a highly versatile biosensor for food allergen detection and prediction [6]. However, the modified processes of electrode still limit the application of these kinds of methods. A laser particle size analyzer is a conventional instrument for analyzing the size of nanoparticles that is widely applied. Mast cell degranulation is a process that releases granules into extracellular spaces; the ‘released granule’ retains its shape after exocytosis and exists as a discrete particle in the extracellular space [7]. Nanoparticle analysis techniques have been used to study the characteristics of particles from various original samples in recent years due to their involvement in cell-to-cell signaling and their utility as disease markers [8,9]. However, there is still no established method to measure mast cell degranulation via directly examining granule particle release.

In the present work, we demonstrate for the first time that particle analysis, based on the laser particle size analyzer, can be developed as a much simpler, more rapid and sensitive method for mast cell degranulation detection than the established methods, which are currently widely applied. By directly evaluating the particles in the cell medium of RBL-2H3 cells, a cell line that is widely used as a convenient model to study the degranulation of mast cells and basophils [10], we found that changes of the particle intensity could be used as a new indicator to assess the degranulation level of the activation of mast cells and basophils. Based on this finding, a simple, rapid, sensitive and low-cost strategy was developed, and the method was successfully employed as a cell-based biosensor for screening allergenic chemical agents in the real environment. This method not only monitors compound 48/80 (C48/80), ionomycin and sinomenine (SIN) induced RBL-2H3 cell degranulation, which could be assessed by the β-hexosaminidase assay, but also quantifies the intensity of the RBL-2H3 cell degranulation events, which remains undetected by the β-hexosaminidase assay. Collectively, the method is expected to be developed into a potential method for rapid mast cell degranulation detection and could further be used for screening allergenic chemical agents in mast cells, animal models and clinical allergic responses.

2. Materials and methods

2.1. Cell line and reagents

Rat basophilic leukemia-2H3 basophils (RBL-2H3) were purchased from the American Type Culture Collection (ATCC, #CRL-2256). The cells were cultured in dulbecco’s modified eagle’s medium (DMEM, GIBCO, Big Cabin, Oklahoma, ME, USA) supplemented with 10% fetal bovine serum (FBS, GIBCO, Clontech, Mountain View, CA, USA) and 100 U/ml penicillin and 100 U/ml streptomycin (Invitrogen, Scotland, UK) at 5% CO2 and 37 °C in a humidified incubator. Compound 48/80 (C48/80), 4-nitrophenyl N-acetyl-β-D-glucosaminidase and ionomycin were obtained from Sigma. Sinomenine (>98% purity verified by HPLC) was provided by the Hunan Zhengqing Pharmaceutical Co. Ltd (Hunan, China).

2.2. Exocytosis stimulation

RBL-2H3 cells were seeded at a density of 1 x 10^5 cells/ml in 35 mm dishes. After stabilization overnight, the RBL-2H3 cells were spun at 1500 rpm and washed with Tyrode’s buffer (135 mM NaCl, 5 mM KCl, 1.8 mM CaCl2, 1.0 mM MgCl2, 5.6 mM Glucose, 20 mM HEPES, and 1 mg/mL BSA at pH 7.4). The cells were then treated with C48/80 (30 μg/ml) or ionomycin (10 μM) in Tyrode’s buffer for the indicated times at 37 °C. The exocytosis processes of the cells were recorded, and the morphology of cells was captured using a Leica microscope equipped with a 5 Megapixel HD Microscope Camera. (Leica MCI70 HD, Life science, German).

2.3. Measurement of β-hexosaminidase release

The degranulation response of the RBL-2H3 basophils was quantified by measuring the level of β-hexosaminidase released in the supernatants according to a previously described method [11]. In brief, RBL-2H3 cells were seeded into 96-well plates. After stabilization overnight, the cells were washed with Tyrode’s buffer (135 mM NaCl, 5 mM KCl, 1.8 mM CaCl2, 1.0 mM MgCl2, 5.6 mM Glucose, 20 mM HEPES, and 1 mg/mL BSA at pH 7.4) three times and were stimulated with C48/80 (30 μg/ml), ionomycin (10 μM) or sinomenine (100 μM) for different time points. The plate was then centrifuged at 1000 rpm for 5 min, and 30 μL of the supernatant from each well was transferred to another flat-bottom plate for enzyme activity analysis.

Twenty microliters of the supernatant was mixed with 20 μL of the substrate solution (4 mM 4-nitrophenyl N-acetyl-β-D-glucosaminidase in 0.1 M citrate buffer at pH 4.5) in 96-well plates and was incubated for 1 h at 37 °C. The reaction was then terminated by the addition of 200 μL of 0.2 M Glycine at pH 10.7. The absorbance was measured at 405 nm (Infinite M200PRO microplate spectrophotometer, TECAN, USA). The results of the cell degranulation are expressed as the percentage of degranulation relative to the control sample.

2.4. Measurement of intracellular Ca^{2+} levels

The intracellular Ca^{2+} levels of the RBL-2H3 basophils were detected according to a previously described method [12]. Briefly, RBL-2H3 cells were incubated with compound C48/80 at 30 μg/ml or ionomycin at 10 μM for the indicated time points. The cells were harvested in a cell dissociation solution and washed with a balanced salt solution (BSS) (148 mM NaCl, 49 mM KCl, 63 mM d-sorbitol, 2.63 mM K2HPO4, 3H2O, 1 mM KH2PO4, and 10 mM HEPES). After being centrifuged at 1500 RPM for 5 min, the cells were resuspended at a density of 5 x 10^6/ml in BSS: Unlabeled cells were served as a negative control, and the calcium probe Fluo-3AM (BD Bioscience, USA) was added to the sample at a final concentration of 5 μM. The cells were incubated with Fluo-3AM for 30 min at 37 °C in the dark and were then washed twice with BSS. The cells were analyzed on a BD FACs analyzer. Fluo-3AM fluorescence was monitored at an excitation wavelength of 488 nm.

2.5. Blood collection and plasma preparation

Female Sprague–Dawley rats (8 weeks) were purchased from the Guangdong Medical Laboratory Animal center. The animals were housed in a laminar air flow room maintained at a temperature of 22 ± 2 °C and a relative humidity of 55 ± 5% throughout the study. The care and treatment of the animals were in accordance with the Laboratory Animal Research Committee Guidelines.
Fig. 1. C48/80 and ionomycin induced RBL-2H3 cell degranulation. (A) C48/80 and (B) ionomycin induced cellular morphological changes of RBL-2H3 cells. (C) C48/80 and (D) ionomycin induced β-hexosaminidase release in RBL-2H3 cells. All data are expressed as the mean ± standard error of mean (S.E.M.) of three or more independent experiments. **p < 0.01, ***p < 0.001 compared with vehicle control.

Fig. 2. Effects of C48/80 and ionomycin on the intracellular Ca²⁺ level in RBL-2H3 cells determined by flow cytometry. (A) C48/80 and (B) ionomycin were employed to stimulate RBL-2H3 cells for 5 min, 30 min or 120 min or for 5 min, 10 min, or 30 min. The results are representative of three independent experiments.
Fig. 3. C48/80 and ionomycin induced RBL-2H3 cell degranulation determined by nano-sight particle analysis method. Particle intensity (A-B) and statistical analysis (C-D) of the released granules from Tyrode’s buffer of C48/80 and ionomycin stimulated RBL-2H3 cells. Statistical analysis of the released particle size from Tyrode’s buffer of (E) C48/80 and (F) ionomycin stimulated RBL-2H3 cells. All data are expressed as the mean ± standard error of mean (S.E.M.) of three or more independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001 compared with vehicle control.
of Guangzhou University of Traditional Chinese Medicine. The rats were injected with PBS with or without 200 mg/kg sinonemine. Blood was collected at the end of the experiments, and plasma was prepared according to the previous description [13]. Briefly, the blood was centrifuged at 2000g for 10 min at 20 °C without using a brake, and then the plasma was carefully collected and centrifuged again at 2000g for 10 min at 20 °C without using a brake. The plasma samples were aliquoted in 200 μl portions for further analysis. For the isolation of the nanoparticles from the plasma, 200 μl of the sample was centrifuged at 18890g at 20 °C for 30 min with a minimum brake. The supernatants were carefully removed, except for the nearly 20 μl containing the particle pellet.

2.6. Particle analysis assay

RBL-2H3 cells were seeded into a 96-well plate. After stabilizing overnight, the cells were washed with Tyrode’s buffer or not. The cells were stimulated with C48/80 (30 μg/ml), ionomycin (10 μM) or sinonemine (100 μM) in Tyrode’s buffer or culture medium for different time points. The plate was then centrifuged at 10000g for 10 min, and 100 μl of the supernatant from each well was transferred to another flat-bottom plate for particle analysis. The cell supernatants were then diluted in ultrapure water, and the plasma was diluted in ddH2O at ratios of 1:9 and 1:99. The samples were analyzed using a Nanosight NS300 in a light scatter mode according to a previous description with a few modifications [14]. The nanoparticle tracking analysis software defined the concentration, size and intensity of the particles within the samples. The particle distribution was measured using dynamic light scattering (DLS) on a Malvern Zetasizer Nano-ZS (Malvern Instruments, Worcestershire, UK) as described previously [15]. The intensity of the size distribution was determined at 25 °C. Each sample was analyzed in triplicate, and each replicate was measured six times.

2.7. Statistical analyses

The differences were considered statistically significant when the p-value was less than 0.05. Statistical comparisons between the groups were performed by a one-way analysis of variance (ANOVA) followed by Tukey’s or Bonferroni’s method as a post-hoc test.

3. Results and discussion

3.1. Determination of RBL-2H3 cell degranulation induced by C48/80 and ionomycin using a β-hexosaminidase assay

Mast cells play very important roles in the initiation and control of allergic inflammation, which is associated with the release of granules that store inflammatory mediators [16]. C48/80, a typical polybasic compound capable of causing the degranulation of mast cells, is widely used as a positive control for anaphylaxis research in mast cells [17]. Ionomycin, which is produced by the bacterium Streptomyces Conglobatus, is a mobile ion carrier for Ca2+ that also induces mast cell degranulation [18]. In the present study,
both C48/80 and ionomycin are served as positive agents to stimulate RBL-2H3 cell degranulation. We first determined the effects of C48/80 and ionomycin on the morphological alteration and β-hexosaminidase release in RBL-2H3 cells. It was showed that the cellular morphology was changed significantly, and granule-like particles appeared on the surface or surrounding the RBL-2H3 cells after C48/80 and ionomycin stimulation (Fig. 1A and B). The real-time morphological changes of the RBL-2H3 cells upon C48/80 or ionomycin stimulation also indicated the presence of granule-like particles on the surface of or surrounding the RBL-2H3 cells (Supplementary video). The results of the β-hexosaminidase assay also demonstrated that both C48/80 and ionomycin increased β-hexosaminidase release from the RBL-2H3 cells in Tyrode’s buffer (Fig. 1C and D). These results imply that both C48/80 and ionomycin could significantly induce mast cell degranulation, and could be used for the following particle analysis to detect mast cell degranulation.

3.2. Analysis of RBL-2H3 cell degranulation concomitant with increasing intracellular Ca\(^{2+}\) levels induced by C48/80 and ionomycin

It was reported that calcium induces the extrusion of secretory granules (exocytosis) in mast cells exposed to C48/80 and ionomycin [19,20]. In our previous study, we demonstrated that both C48/80 and ionomycin could trigger RBL-2H3 cells to release β-
hexosaminidase, which was also in parallel with degranulation (exocytosis) [21]. In the present study, we measured the intracellular Ca\(^{2+}\) level by flow cytometry to evaluate its influence on secretory granules (exocytosis). RBL-2H3 cells were treated with the reference compounds C48/80 and ionomycin for the indicated time points. Our data showed that the two reference compounds obviously increased the intracellular Ca\(^{2+}\) levels (Fig. 2A and B), which are coincided with our previous report [21]. These results provide intensive evidence for the hypothesis that the release of β-hexosaminidase is closely consistent with particle secretion. Therefore, we inferred that particle analysis has the potential to be developed as a new method to assess cell degranulation that is induced by agents or drugs.

### 3.3. Qualitative analysis of mast cell degranulation by the particle analysis assay

In mast cells, the preformed inflammatory mediators histamine and β-hexosaminidase are stored in granules. Granules can be released from the cell by the process of exocytosis, and some of the stored inflammatory mediators are released, while others are contained in the granules and slowly released along the location of the granules [22,23]. Because the ‘released granule’ retains its shape after exocytosis and exists as a discrete particle in the extracellular space after degranulation [7], we assumed that mast cell degranulation can be detected by the direct measurement of the released granules. Nanosight NS300, an instrument for measuring the particle size that is based on the Brownian movement of nanoparticles, was employed to detect the particles in the supernatants of the RBL-2H3 cells after C48/80 and ionomycin stimulation (in Tyrode’s buffer). Interestingly, the intensity of the particles was prominently enhanced after stimulation with C48/80 and ionomycin (Fig. 3A and B). These results provide direct visual images to demonstrate mast cell degranulation. Unfortunately, this instrument does not provide quantified data of the degranulation, and we further quantified the intensity of the particles using professional software that is usually used to analyze Western blotting results. Statistical analysis also demonstrated that C48/80 and ionomycin significantly increase the intensity of the particles in the supernatants of the RBL-2H3 cells (Fig. 3C and D). Moreover, the average size of the particles, ranging from 100 to 200 nm, was also remarkably increased with C48/80 and ionomycin stimulation (Fig. 3E and F). These data provide solid evidence for the assumption that the released particles can be determined by directly detecting the particle intensity or the particle size to investigate mast cell degranulation.

### 3.4. Quantitative analysis of mast cell degranulation by the particle analysis assay

Secretory granules with an average size of 100 nm were originally reported to have dense cores and store different types of inflammatory mediators, including β-hexosaminidase and histamine [24–26]. Although intensity analysis of the released particles by RBL-2H3 cells provides some visual data to examine mast cell degranulation, the distribution of the particle size needs to be investigated. The Zetasizer Nano ZS-90 was used to quantify the size of the particles released from the mast cell degranulation. As shown in Fig. 4A-B, the size of the particles in the supernatants of the control RBL-2H3 cells was mainly concentrated at an average of 10 nm. However, after C48/80 and ionomycin stimulation (in Tyrode’s buffer), the particles in the supernatants of the RBL-2H3 cells mainly shift to the range of 100–200 nm. Furthermore, the percentage of particles located in the 100–200 nm range was enhanced with the increased stimulation time (Fig. 4A and B). Because the Zetasizer Nano ZS-90 provides the intensity percentage of the particles in each peak in the results, the increased particles ranging from 100 to 200 nm can be quantified to demonstrate mast cell degranulation. The intensity percentage of the particles in the 100–200 nm range was also increased.
range for the control group were normalized as 100%, and other groups were expressed as the percentage of the control samples. As shown in Fig. 4C and D, the percentage of particles ranging from 100 to 200 nm was dramatically increased with C48/80 and ionomycin treatment in a time-dependent manner in accordance with β-hexosaminidase release. These results suggest that the percentage of the particles ranging from 100 to 200 nm in the supernatants of the RBL-2H3 cells could be used as a new indicator to quantitatively assess the level of degranulation of the mast cells.

3.5. Quantitative analysis of mast cell degranulation in the cell culture medium by the particle analysis assay

The β-hexosaminidase assay is a colorimetric assay, which means that the drug stimulation of RBL-2H3 cells cannot be used in the cell culture medium as the color of the medium would interfere the results. Therefore, the C48/80 and ionomycin stimulation was finished in Tyrode's buffer. As shown in Fig. 5A and B, the β-hexosaminidase assay and the established particle analysis assay showed parallel results in Tyrode's buffer, demonstrating mast cell degranulation at different cell numbers upon C48/80 stimulation. Furthermore, the particle analysis assay was more sensitive than the conventional β-hexosaminidase assay when the number of cells decreased to 500.

It is well known that medium is widely used for cell culture due to its appropriate nutrition and environment for cell growth. Although the β-hexosaminidase assay is very useful for detecting mast cell degranulation upon a short stimulation with drugs or chemical agents, the long-term exposure of cells in Tyrode's buffer disturbs the normal physiological status of cells and in turn affects the results. Therefore, an important advantage needed to be determined whether the particle analysis assay can be directly investigated in cell culture medium for investigating mast cell degranulation. As indicated in Fig. 5C, the results demonstrate that mast cell degranulation in cell culture medium can also be successfully evaluated by the particle analysis assay. Collectively, the current results reveal that this particle analysis assay is more convenient and sensitive than the widely used β-hexosaminidase assay, especially when it is used to detect mast cell degranulation in cell culture medium. Based on the characteristics and advantages of this method, the particle analysis assay is expected to be used for the real-time and in-situ detection of mast cell degranulation in cell culture medium.

3.6. More sensitive analysis of mast cell degranulation by the particle analysis assay

The particle analysis assay not only provides a simpler and more rapid method than the β-hexosaminidase assay for mast cell degranulation detection, but it can also provide information on the physiological state of cells, which cannot be obtained using the β-hexosaminidase assay. We further compared the sensitivity of the particle analysis assay and the β-hexosaminidase assay through evaluating degranulation in different number of mast cells. For the β-hexosaminidase assay, the degranulation of 400 RBL-2H3 cells stimulated by C48/80 was clearly identified, but when the cell num-

Fig. 8. Plasma from sinomenine induced systemic anaphylaxis in rats determined by particle analysis method. (A) Particle intensity analysis of the plasma from rats. (B) Particle distribution analysis of the plasma from rats. (C) Particle intensity, particle size and particle concentration analysis from the plasma of rats. All data are expressed as the mean ± standard error of mean (S.E.M.) of three or more independent experiments. "p < 0.01, ""p < 0.001 compared with vehicle control.
ber decreased to 300 cells or fewer, the degranulation of RBL-2H3 cells induced by C48/80 was not detected by using the traditional β-hexosaminidase assay (Fig. 6A). However, the particle analysis assay detected RBL-2H3 cell degranulation even when only 20 RBL-2H3 cells were stimulated by C48/80 (Fig. 6B), suggesting that the particle analysis assay is more sensitive than the β-hexosaminidase assay for the detection of mast cell degranulation.

3.7. Analysis of drug-induced mast cell degranulation in vitro by the particle analysis assay

Because immunocyten and C48/80 induced mast cell degranulation is easily determined by the particle analysis assay, we further investigated whether the particle analysis assay can also be employed to detect mast cell degranulation induced by a clinical drug, sinomenine, which is a widely used drug against rheumatoid arthritis derived from the Chinese medicinal plant Sinomenium acutum with allergy occurring according to clinical reports [27,28]. In the study, sinomenine induced RBL-2H3 cell degranulation could be determined by the particle analysis assay and the β-hexosaminidase assay in Tyrode’s buffer. As shown in Fig. 7A and B, RBL-2H3 cell degranulation induced by sinomenine could be monitored by both assays, which is consistent with our previous results [29]. Additionally, sinomenine-induced RBL-2H3 cell degranulation was detected by the particle analysis assay in cell culture medium (Fig. 7C). It was reported that the degranulation of mast cells occurs in a calcium-independent manner that is different from our reference compounds, such as the sinomenine–induced degranulation of RBL-2H3 cells [12,30]. These results indicate that our newly established particle analysis assay can be effectively used to investigate drugs or chemical agents that induce mast cell degranulation responses in different mechanisms with high sensitivity.

3.8. Analysis of drug-induced systemic anaphylaxis in an in vivo rat model by the particle analysis assay

Although the β-hexosaminidase assay is classically used for the determination of degranulation, it is limited to determine mast cell degranulation in blood samples. To demonstrate the outstanding ability of the particle analysis assay for more complicated sample detection, we analyzed blood samples from passive systemic anaphylaxis rats induced by sinomenine (Fig. 8A). We found that the percentage of the particles ranging from 100 to 200 nm increased in the plasma from the sinomenine–treated animals (Fig. 8B). Moreover, the particle size and the particle concentration were remarkably increased in the sinomenine-treated rats compared to the vehicle treatment (Fig. 8C). These results demonstrate the ability of the established particle analysis assay for blood sample detection, showing that the method has the potential for screening allergic effects in vivo. Based on these results, the particle analysis assay is expected to be developed into a promising method for clinical blood sample detection to determine anaphylaxis responses in patients.

4. Conclusion

In summary, a rapid, sensitive and low-cost method for mast cell degranulation detection was established. This particle analysis based strategy can be used to monitor mast cell degranulation by direct measurement of the released granules, which is in accordance with results from a traditional β-hexosaminidase assay. More importantly, this particle analysis assay can be directly performed in cell culture medium without complicated sample preparation procedures, which cannot be obtained using the traditional β-hexosaminidase assay. Most importantly, the currently established particle analysis assay is very sensitive and can detect mast cell degranulation in small numbers of cells, such as 20 cells, whereas the conventional β-hexosaminidase assay cannot detect mast cell degranulation when there are fewer than 300 cells. This particle analysis assay can be used to detect drug-induced mast cell degranulation in vitro and passive systemic anaphylaxis in vivo models. Collectively, the findings highlight the applications of this particle analysis assay as a highly sensitive and rapid method for mast cell degranulation detection, which is expected to be developed into a promising assay to screen drugs and chemical agents both in vitro and in vivo models, and will even be developed to detect anaphylaxis responses in clinical samples in the future.

Conflict of interests

The authors declare that they have no conflict of interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phrs.2016.05.033.

References


