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The effects of imidacloprid combined with endosulfan on IgE-mediated mouse bone marrow-derived mast cell degranulation and anaphylaxis

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ABSTRACT

Low levels of endosulfan are known to stimulate mast cells to release allergic mediators, while imidacloprid can inhibit IgE-mediated mast cell degranulation. However, little information about the effects of both pesticides together on mast cell degranulation is available. To measure the effects, IgE-activated mouse bone marrow-derived mast cells (BMMCs) were treated with imidacloprid and endosulfan, individually, and simultaneously at equi-molar concentrations in tenfold steps ranging from 10^{-4} to 10^{-11} M, followed by measuring several allergy-related parameters expressed in BMMCs: the mediator production and influx of Ca^{2+}, the phosphorylation content of NF-κB in the FcεRI signaling pathway. Then, the effects of the mixtures on IgE-induced passive systemic anaphylaxis (PSA) of BALB/c was detectded. This study clearly showed that the application of equi-molar mixtures of both pesticides with 10^{-4}–10^{-5} M significantly inhibited the IgE-mediated mouse bone marrow-derived mast cells degranulation *in vitro* and 10^{-4} M of them decreased IgE-mediated PSA *in vivo*, as the application of imidacloprid at the same concentration alone did. Morever endosulfan alone had no remarkable stimulatory effects on any of the factors measured. In conclusion, simultaneous application of equi-molar concentrations of both pesticides generally showed highly similar responses compared to the responses to imidacloprid alone, suggesting that the effects of the mixture could be solely attributed to the effects of imidacloprid.

1. Introduction

Pesticides are the largest group of chemicals that are used widely in modern agricultural practices for crop protection, and pesticide-related untoward health effects in humans, animals and birds have become a serious threat to human health [1,2]. Many pesticides were reported to affect the human immune system, possibly contributing to the trend of increasing allergic diseases [3,4].

Although many organochlorine pesticides have been banned, residues of them continue to be found in the environment, foods, and in human tissues [5,6]. Among them, endosulfan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9 a-hexahydro-6,9-methano- 2,4,3- benzodioxathiepin-3-oxide), an organochlorine insecticide, is known as a persistent organic pollutants (POPs) in 2011 [7]. Its residues have been detected in soil, water, various fruits, vegetables, nuts, grains, fish and milk [8–10]. Endosulfan may have adverse effects on different human organ systems including the endocrine, reproductive, developmental, cardiovascular and immune system, and is especially known as a strong neurotoxin in insects and mammals including humans [11–14] and leading to be forbidden in European countries since 2005 and in many other countries worldwide. Intake of over 35 mg/kg body weight (*i.e.* ~100 mL of a 10^{-4} M solution) of endosulfan is mortal. However, it was also shown that low levels of endosulfan might stimulate bone marrow-derived mast cells (BMMCs) and human mast cell line HMC-1 to release allergic mediators, suggesting a possible positive association of endosulfan with the development of allergic diseases [15,16].

Imidacloprid, 1[(6-chloro-3-pyridinyl) methyl-N-nitro-2-imidazolidinimine, is a well-known neonicotinoid which acts as a nicotinic-acetylcholine receptor (nAChR) agonist [17]. Human exposure to imidacloprid, and its impact on health may occur due to its extensive use and environmental persistence. Its residues have been found in drinking water and food [18–20]. Tolerances for imidacloprid residues in food range from 0.02 mg/kg in eggs to 3.0 mg/kg on hops [21]. Recent advances showed that imidacloprid can affect adaptive and inflammatory immune responses of rats [22–24]. Our recent studies indicated that imidacloprid can inhibit mast cell degranulation, suggesting a possible

Abbreviations: BMMCs, bone marrow-derived mast cells; PSA, passive systemic anaphylaxis; nAChR, nicotinic-acetylcholine receptor

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negative association or antagonistic activity of imidacloprid in allergic reactions [25].

In the past, much emphasis was placed on evaluating the possible effects of single pesticide on health, whilst it is now increasingly recognized that simultaneous exposure of humans to different chemicals including pesticides from various sources and with different but specific immunological effects has become a reality, and that there are more attention being given in health care science [26–28]. Therefore, in this study, the combined effects of both specific pesticides on mast cell was tested in IgE-activated bone marrow-derived mast cells (BMMCs), treated with equi-molar mixtures of endosulfan and imidacloprid. We measured the ratio of degranulated mast cell, the Ca^{2+} influx, the release of allergic mediators and the phosphorylation contents of NF-κB in FcεRI signaling pathway, and the IgE-induced passive systemic anaphylaxis (PSA) using Evans blue extravasation, serum levels of histamine, LTC4 and TNF-α. Accordingly, the aim of this work was to explore the possible antagonistic relationship between imidacloprid and endosulfan in relevant factors of IgE-mediated allergic diseases.

2. Materials and methods

2.1. Mice and reagents

BALB/c mice were purchased from the Department of Animal Science, Nanchang University (Nanchang, China) at the age of 8 weeks. All animal-use protocols were approved by the Animal Care Committee under the guidelines of the Care and Use of Laboratory Animals (NIH Publication No. 86-23, Revised 1996).

Imidacloprid, endosulfan, ketotifen fumarate salt, and monoclonal anti-dinitrophenyl IgE antibody (anti-DNP IgE) were obtained from Sigma-Aldrich Corporation (St. Louis, MOUSA). DNP–HSA was obtained from Biosearch Technologies, Inc. (Novato, CA,USA). Antibodies against phosphorylated forms of NF-κB and antibody against total NF-κB were purchased from Cell Signaling Technology (Danvers, Mass). Cell culture medium (RPMI 1640 medium, 1640) and other reagents were purchased from Thermo Fisher Scientific (Waltham, Mass).

2.2. BMMCs

BMMCs were generated from bone marrow cells of BALB/c mice as described before [29]. Briefly, bone marrow cells were harvested from the femoral bone marrow and cultured in complete RPMI medium supplemented with recombinant mouse IL-3 (30 ng/mL; R&D Systems) and recombinant mouse stem cell factor (50 ng/mL; R&D Systems, Abingdon, UK). After four weeks later, the differentiation of BMMCs was monitored by measuring the expression of FITC-anti-mouse c-Kit and PE-anti-mouse FcƐRI (eBioscience, Inc., San Diego CA, USA) by flow cytometry. The FACS analysis showed that > 80% of the cells were CD117 cells (c-kit) and FcƐRI positive.

2.3. Treatment of BMMCs

The BMMCs were sensitized with 500 ng/mL anti-DNP IgE overnight at 37 °C. Then, the cells were treated with mixtures of endosulfan and imidacloprid at equi-molar concentrations (ranging from 10^{-4} to 10^{-11} M) for 4 h, followed by the stimulation with 50 μg/mL DNP–HSA. Cells and supernatants were collected and subjected to various assays. Pure culture medium was used as negative control; ketotifen fumarate at 10^{-5} M was used as positive control.

2.4. Assessment of degranulation

After the cells were stimulated with DNP–HAS for 30 min, the percentage of degranulated mast cells was calculated from 1100 cells analyzed by microscopic examination in three independent replicates. Cells without granules were considered degranulated. The released histamine was measured by enzyme immunoassay according to the manufacturer's instructions (Cayman Chemical). The amount of histamine release into media was expressed as the percentage of the total amount of histamine originally in the cells.

2.5. Assessment of LTC4 and TNF-α

The supernatants were collected after BMMCs were stimulated with DNP–HAS for 4 h. The released eicosanoids were evaluated by testing the content of LTC4 in the supernatants using immunoassay kits from Cayman Chemical (Ann Arbor, MI). The production of cytokines was assessed by quantifying the content of TNF-α in the supernatants using an ELISA kit from Abcam, Inc. (Cambridge, MA, USA).

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

To analyze intracellular Ca^{2+} levels, the fluorescence assay was performed as previously described [30]. After treatments with equimolar mixtures of endosulfan and imidacloprid, about 1×10^6 BMMCs were pre-incubated with 5 μM Fluo-3/AM containing 0.05% Pluronic F127 for 30 min before adding 50 μg/mL DNP–HSA. The fluorescent intensity was measured at 488 nm excitation wavelength and 526 nm emission wavelength by a Varioskan Flash microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

2.7. Western blot analysis of NF-κB

Crude nuclear extract was prepared as previously described [31]. Protein concentration was determined using the Bicinchoninic acid method. The proteins were resolved by 15% SDS-PAGE and subsequently electrotransfered to a nitrocellulose membrane. Blots were blocked with 2% bovine serum albumin (Sigma–Aldrich) blocking buffer for 1 h at room temperature and then probed with NF-κB antibody overnight at 4 °C. After washing three times with TBS-T, the membranes were incubated with a secondary antibody for 1 h at room temperature. Afterward, the immune reaction was visualized by the enhanced chemiluminesence detection system (Bio-Rad, Hercules, USA), followed by digitizing the band intensity using Quantity One software.

2.8. Passive systemic anaphylaxis in mice

Passive systemic anaphylaxis (PSA) was done as described previously [32]. BALB/c mice were injected intraperitoneally with 2 mg of anti-DNP IgE in 100 μL saline. After 24 h, the mice were treated intraperitoneally with a mixture of imidacloprid and endosulfan at equimolar concentrations in 100 μL DMSO for 4 h, followed by an intravenously challenge with 2 mg of DNP-HSA in 100 μL of saline containing 4% Evans blue. Evans blue extravagated by the antigen was extracted overnight at 63 °C with formamide and measured by absorbance at 630 nm. Blood was collected by cardiac puncture 30 min or 4 h after the DNP-HSA challenge. Concentrations of serum histamine, LTC4 and TNF-α were determined as described above.

2.9. Statistical analysis

All experiments were performed at least three times independently using new batches of cells, with each batch including about 1×10^6 cells. Statistical comparisons between groups were performed using unpaired two-sided *t*-tests. Differences at $p < 0.05$ were considered significant.