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Detection of 3-phenoxybenzoic acid in river water with a colloidal gold-based lateral flow immunoassay

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ABSTRACT

3-Phenoxybenzoic acid (3-PBA) is a general metabolite of synthetic pyrethroids. It could be used as a generic biomarker for multiple pyrethroids exposure for human or pyrethroid residues in the environment. In this study, monoclonal antibodies (mAbs) against 3-PBA were developed by using PBA–bovine serum albumin (BSA) as an immunogen. In the competitive enzyme-linked immunosorbent assay (ELISA) format, the I_{50} and I_{10} values of purified mAbs were 0.63 and 0.13 μg/ml, respectively, with a dynamic range between 0.19 and 2.04 μg/ml. Then, the colloidal gold (CG)-based lateral flow immunoassay was established based on the mAbs. The working concentration of coating antigen and CG-labeled antibodies and the blocking effects were investigated to get optimal assay performance. The cutoff value for the assay was 1 μg/ml 3-PBA, and the detection time was within 10 min. A total of 40 river water samples were spiked with 3-PBA at different levels and determined by the lateral flow immunoassay without any sample pretreatments. The negative false rate was 2.5%, and no positive false results were observed at these levels. This lateral flow immunoassay has the potential to be an on-site screening method for monitoring 3-PBA or pyrethroid residues in environmental samples.

Due to their low mammalian toxicity and efficient control of insects, pyrethroids are one of the most widely used insecticides in agriculture and private households [1,2]. However, increasing use of pyrethroid insecticides has resulted in concerns regarding potential effects on the ecosystem and human health. In addition to their high toxicity to aquatic life [3], some research also indicates that pyrethroids exposure may cause developmental neurotoxicity [4], adverse affects of male reproduction [5], and endocrine system disruption [6].

3-Phenoxybenzoic acid (3-PBA)[1] is a general metabolite of a number of common synthetic pyrethroids (Fig. 1) such as cypermethrin, deltamethrin, permethrin, cyhalothrin, fenvalerate, fenpropathrin, sumithrin, and possibly other pyrethroid insecticides [7–9]. Thus, 3-PBA is commonly used as a generic biomarker for multiple pyrethroids exposure for humans [8,10]. In the environment, 3-PBA is more mobile and persistent than its parental compounds and has widespread occurrence in surface water, sediment, and soil; the detection of 3-PBA may also reflect the multiple residues of pyrethroid insecticides [2,11,12].

Several chromatographic methods have been reported for the analysis of 3-PBA in different matrices [13–16]. More recently, enzyme-linked immunosorbent assays (ELISAs) and other bioanalytical approaches have been established as alternative methods for exposure monitoring with a detection limit of ppb (parts per billion) levels [17–20]. However, most of them need to be used in laboratories and operated by trained personnel.

The colloidal gold (CG)-based lateral flow immunoassay is a popular and user-friendly format in terms of simplification and rapid on-site testing. It can be rapidly completed in one step, and the results can be observed with naked eyes [21]. To our best knowledge, this is the first report for development a CG-based lateral flow immunoassay for 3-PBA and its application to river water samples.

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[1] *Abbreviations used:* 3-PBA, 3-phenoxybenzoic acid; ELISA, enzyme-linked immunosorbent assay; CG, colloidal gold; BSA, bovine serum albumin; OVA, ovalbumin; KLH, keyhole limpet hemocyanin; mAb, monoclonal antibody; NC, nitrocellulose; CBS, carbonate–bicarbonate buffer saline; PBS, phosphate-buffered saline; PBST, PBS containing Tween 20; CPBS, citrate–acetate buffer; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TMB, tetramethylbenzidine; TEM, transmission electron microscope.

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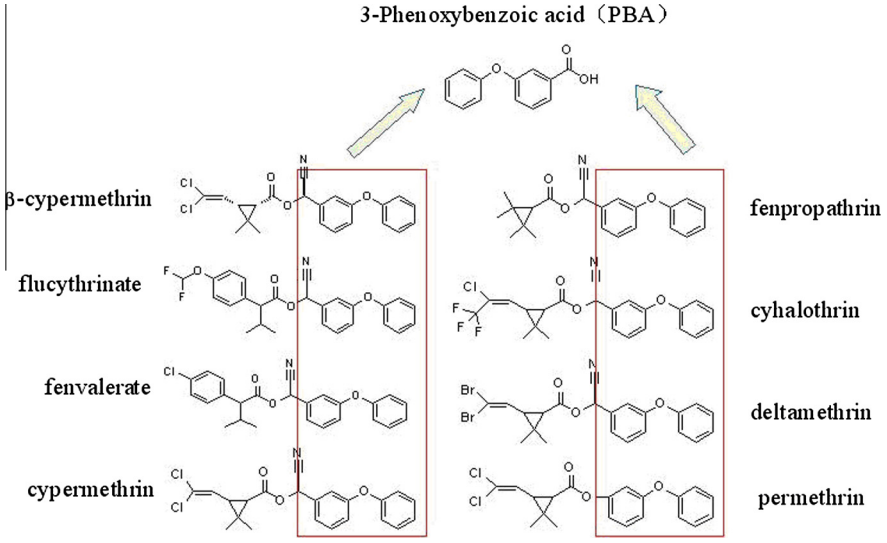

Fig.1. Chemical structures of pyrethroid pesticides and 3-phenoxybenzoic acid (3-PBA).

Materials and methods

Chemicals and buffers

3-PBA, bovine serum albumin (BSA), ovalbumin (OVA), keyhole limpet hemocyanin (KLH), Hybri-Max polyethylene glycol solution (average molecular weight = 1450), complete and incomplete Freund's adjuvants, and mouse monoclonal antibody (mAb) isotyping reagents were obtained from Sigma–Aldrich (St. Louis, MO, USA). Murine myeloma cells (Sp2/0-Ag14) were a gift from Xinxia Xia (Institute of Veterinary Medicine, Jiangsu Academy of Agricultural Sciences, China). HiTrap Protein G HP columns were obtained from GE Healthcare (Piscataway, NJ, USA). Peroxidase-labeled goat anti-mouse IgG was obtained from KPL (Gaithersburg, MD, USA). RPMI 1640 medium and HT and HAT supplements were obtained from Life Technologies (Carlsbad, CA, USA). Fetal bovine serum was obtained from Boster (Wuhan, China). Nitrocellulose (NC) membranes (HF135), sample pads, absorbing pads, and CG pads were obtained from Millipore (Bedford, MA, USA). All other chemicals used in the current study were of analytical grade. River water samples were collected from local rivers of Nanjing, China.

Carbonate–bicarbonate buffer saline (CBS: 50 mmol/L, pH 9.6), phosphate-buffered saline (PBS, 50 mmol/L, pH 7.4), PBST (PBS containing 0.05% [v/v] Tween 20), and citrate–acetate buffer (CPBS: 25 mmol/L citrate and 62 mmol/L sodium phosphate, pH 5.5) were used for immunoassay. GB-A solution (0.02 mol/L Tris–HCl [pH 8.6], 0.1% PEG 20,000, 4% sucrose, and 0.02% NaN_3) was prepared to resuspend the CG–mAb conjugates.

Protein–hapten conjugates and immunization

3-PBA was used as the hapten, and PBA–BSA and PBA–OVA were synthesized by using the active ester method and mixed anhydride method, respectively [22].

Four 6-week-old BALB/c female mice each received four 100-μl intraperitoneal injections at 2-week intervals. Injections consisted of a 1:1 emulsion of 100 μg of PBA–BSA in PBS and Freund's adjuvant (complete for the first dose and incomplete for subsequent ones). The titers and affinity of the antiserum were determined by ELISA. Three days before the fusion, the mouse with the highest serum affinity to PBA was boosted with 200 μg of PBA–BSA in 200 μl of PBS.

Production of mAbs

The fusion protocol was based on Köhler and Milstein's standard method with some modifications. Briefly, 2.25×10^8 mouse spleen cells from the immunized mouse and 4.5×10^7 murine myeloma cells (Sp2/0-Ag14) were fused using Hybri-Max polyethylene glycol solution at 37 °C. Fused cells were selected in HAT medium for 10 days, followed by 1 week in HT medium. Hybridomas were then grown in RPMI 1640 medium supplemented with 20% fetal bovine serum. Hybridoma supernatants were double-checked by noncompetitive ELISA and competitive ELISA. The positive clones were subcloned by the limiting dilution method. The immunoglobulin subclass was determined using mouse mAb isotyping reagents. The choosing positive clones were used to produce ascitic fluids and then purified by a protein G column and characterized by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE).

Competitive ELISA procedures

The 96-well plates were coated with 2 μg/ml PBA–OVA (100 μl/well) in CBS and incubated overnight at 4 °C. The plates were washed three times with PBST by automated microplate washer and were blocked by incubating with 1% OVA in PBS (200 μl/well) for 1 h at 37 °C. After the washing step, 50 μl of PBA standards and 50 μl of cell culture or purified mAbs were added. After incubation for 1 h at 37 °C, the plates were washed. Subsequently, 100 μl/well of diluted horseradish peroxidase-labeled goat anti-mouse IgG was added and incubated for 1 h at 37 °C, and after the washing step 100 μl/well of a tetramethylbenzidine (TMB) solution (120 μl of 10 mg/ml TMB–DMSO [dimethyl sulfoxide] and 30 μl of 0.65% [v/v] H_2O_2 diluted with 11.85 ml of CPBS) was added. The reaction was stopped after 15 min by adding 50 μl/well of 2 mol/L H_2SO_4, and absorbance was read by a microplate reader at 450 nm.

Preparation of CG and CG–mAb conjugates

All glassware used in the experiment was soaked in aqua regia and rinsed thoroughly in water prior to use. After 100 ml of 0.01% (w/v) chloroauric acid ($HAuCl_4$) solution was heated to the boiling point, 2 ml of freshly made 1% sodium citrate was added under

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