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A comparative assessment of cytotoxicity of commonly used agricultural insecticides to human and insect cells

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ABSTRACT

The cytotoxic potential of 13 commonly used agricultural insecticides was examined using cell-based systems with three human HepG2, Hek293, HeLa cells and three insect Tn5B1-4, Sf-21, and Drosophila S2 cells. Data showed that (1) an enhancement of some insecticides (e.g. pyrethroids) on cells proliferation; (2) an inhibition of some insecticides on cells viability; (3) various levels of susceptibility of different cells to the same insecticide; and (4) the cell type dependent sensitivity to different insecticides. The degree of cytotoxicity of insecticides on human cells was significantly lower than that on insect cells ($P < 0.05$). Methomyl, even 20 μg/ml, showed little cytotoxicity at 24 h exposure whereas emamectin benzoate possessed the strongest cytotoxic potential in a dose-dependent fashion. The results revealed comparable cytotoxic property of agricultural insecticides against intact cells.

1. Introduction

The application of insecticides as a major contributing factor ensures the increase of agricultural productivity worldwide, but the extensive use to control agricultural insect pests has made multiple insecticides exist and interact in the environment, causing the possibility of intervention and risk on human/animal health (El-Wakeil et al., 2013; Kallel et al., 2007). For example, the exposure to a mixture of agricultural pesticides (dieldrin, lindane, aldicarb, atrazine, metribuzine and endosulfan) for a short period of time altered the immune response in frogs and rendered them more susceptible to pathogens infections (Christin et al., 2004). Prolonged exposure to insecticides even caused neurological system disorders, chromosomal aberration and DNA damage, reproductive and developmental defects, even high risk of severe human disease (Estevan et al., 2014; Franco et al., 2010; Kallel et al., 2007; Li et al., 2015).

Although the toxic potential of insecticides have been examined in oral and/or contact studies using a number of different animal models, including rats, guinea pigs, even dogs and birds, systemic characteristics of insecticides poisoning remain to be defined (Cha et al., 2014; Nieradko-Iwanicka et al., 2015). Pyrethroids have relatively low toxicity for humans, but the neurobehavioral and respiratory metabolisms of pyrethroids haven't been adequately evaluated (Fiedler et al., 2015). Insect growth regulators including juvenile analogs and molting disruptors that regulate the development of pest insects such as metamorphosis, embryogenesis, and reproduction, have no clinical signs of toxicosis and observable impaired effects on human and animals at the administered levels (Mondal and Parveen, 2000; Tomlin, 2009), but dibenzoylhydrazines (DBHs) such as tebufenozide and methoxyfenozide that are regarded with high environmental safety, are moderate inhibitors of P-glycoprotein (P-gp) and can stimulate P-gp ATPase activity in rat brain and human, so the risk of the DBHs on human is in reliance on their rational use (Kanaoka et al., 2013; Miyata et al., 2016). One reason for these phenomena is that many insecticides retain in human tissue with a relatively short half-life, causing the different exposure assessments on insecticidal toxicity to be inconsistent.

There is an increasing need for rapid and easily interpreted *in vitro* assays to screen for possible poisoning of pesticides (Eddleston et al., 2008; Papoutsis et al., 2012). Cell-based systems have proved to be useful for assessing toxicity and specific risk on target organs under chemical exposure, thereby offering high-level integration on interactions of chemicals with intact cells (Li et al., 2003; Mersch-Sundermann et al., 2004; Polláková et al., 2012). According to the procedure of ISO 10993-5 standard, tested material that was incubated for at least 24 h with pre-cultured cells and decreased viability of cultured cells under 70% of the control, is considered cytotoxic (International Organization for Standardization, 2009). To date, systematic studies to present a

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comparative cytotoxicity for agricultural insecticides are too few. Based on intact cell model, this study aimed to bring insight the direct cytotoxic effect of thirteen commonly used agricultural insecticides on human liver, kidney and cervix cell cultures in comparison with insect cell cultures under *in vitro* conditions for presenting tissue specific toxicities.

2. Material and methods

2.1. Chemicals

MTT (3-(4,5-dimethylthiazol-2-yl) − 2,5-diphenyltetrazolium bromide), fetal bovine serum (FBS) were purchased from Sigma-Aldrich (Canada). Dulbecco's modified Eagle's medium (DMEM) and penicillin-streptomycin solution (10,000 units/ml penicillin plus 10,000 μg/ml streptomycin) were from HyClone (Los Angeles, USA). TNM-FH medium and Schneider's Drosophila medium was from Gibco (Grand Island, NY, USA). Permethrin (95% purity), β-cypermethrin (98% purity), bifenthrin(96% purity) and nitenpyram(98% purity) were obtained from Shanghai Institute of Pesticide Research (Shanghai, China). Imidacloprid (96% purity) was from KWIN Joint-stock Co., Ltd (Jiangsu, China). Acetamiprid (95% purity) was from Nanjing Red Sun Co., Ltd (Jiangsu, China). Lufenuron(99% purity) was from Syngenta AG (Basel, Switzerland). Hexaflumuron (97.5% purity) was obtained from Jiangsu Yangnong Chemical Group Co., Ltd. (Jiangsu, China). NK-17 (CAS#: 1203711-48-1, 98% purity) was from Nankai University (Tianjin, China). Methomyl (96.7% purity) was from Sigma Chemical Company (Silicon Valley, USA). Tebufenozide(95% purity) was from Abcam (Cambridge, MA, USA); Emamectin benzoate (99.6% purity) was from Sigma-Aldrich (Canada). Chlorantraniliprole (98% purity) was from Zhengnong Chemical Co., Ltd (Jiangsu, China). For experimental use, a stock solution (1000 μg/ml) of each chemical was made in dimethyl sulfoxide (DMSO) and diluted in culture media to the desired concentrations.

2.2. Cell cultures

Human liver carcinoma HepG2 cell line, human embryonic kidney Hek293 cell line, human cervical carcinoma HeLa cell line, *Trichoplusia ni* ovarian Tn5B1-4 cell line, *Spodoptera frugiperda* ovarian Sf-21 cell line and Drosophila S2 cell line were purchased from Cell Bank, Chinese Academy of Sciences (Shanghai, China). The human cells were cultured in a humidified 5% CO_2 atmosphere at 37 °C in complete DMEM medium, Tn5B1-4 and Sf-21 cells were cultured at 28 °C in TNM-FH medium, and Drosophila S2 cells were grown at 28 °C in complete Schneider's Drosophila medium. All medium were supplemented with 10% fetal bovine serum, 100 U/ml penicillin plus 100 μg/ml streptomycin. When approximately 80% confluent in 25 cm^2 culture flasks (Corning, USA), human cells were harvested by trypsinization and insect cells were collected after gentle pipetting for experimental use.

2.3. Chemical exposure

The cells were harvested and washed twice in 0.1 M phosphate buffered saline (PBS, pH7.4) and then resuspended in fresh medium. Cells suspensions at a density of 1×10^5 cells per ml were seeded in 96-well plates (100 μl per well) and incubated for 24 h, and then the medium was removed and replaced with fresh medium containing 0.1% DMSO for control, or medium containing 1.25, 2.5, 5, 10 or 20 μg ml^{-1} test insecticide. After a further 24 h incubation period, the cells were used for the viability assays. Four replicates were used for each concentration of an insecticide and for the control.

2.4. Cell viability assay

Cell viability was determined using the MTT assays in which, on reduction by dehydrogenases in metabolically active cells, produces violet-blue formazan crystals, and the amount of MTT formazan is directly proportional to the number of living cells (Stockert et al., 2012; Van Meerloo et al., 2011). Briefly, the chemical-exposed cells were washed twice with PBS and then incubated in 20 μl medium containing 100 mg/ml of MTT at 37 °C for 4 h. After the medium containing MTT was removed, 150 μl of DMSO was added into each well and then the micro-plates were placed on the microplate shaker to dissolve the formazan crystals at room temperature for 10 min. Optical density (OD) was measured with double wavelengths at 492 and 630 nm in a multifunctional microplate reader (Synergy Neo, BioTek, USA). Percent cell viability relative to the control was calculated by using formulas as cell viability (%) = $(OD_{treatment}/OD_{control}) \times 100$. Independent experiments were performed three times.

2.5. Assays on persistent cytotoxicity

The persistent cellular cytotoxicity of emamectin benzoate that showed the strongest potential in inhibiting cell viability in above MTT assays was further measured on HeLa and Tn5B1-4 cells, which were selected randomly from the test cells. After treated by emamectin benzoate for 48 h, The cells, along with the control, were washed twice with PBS, and then transferred to fresh chemicals-free TNM-FH or DMEM medium containing 10% FBS for next 24, 48, and 72 h incubation. The changes in cell proliferation were determined using the MTT assays for cell viability to assess the persistent cellular toxicity of emamectin benzoate.

2.6. Statistical analysis

Data are presented as the mean ± standard errors (*S.E.*). Statistical significance of differences was evaluated by a one-way analysis of variance (ANOVA), followed by least significant difference (LSD) post hoc test analysis. Statistical probability (P) at 0.05 levels was considered significant. Data were analyzed using SPSS 17.0 for windows.

3. Results

3.1. The cytotoxicity of pyrethroids on cells

The exposure to pyrethroids including permethrin, β-cypermethrin and bifenthrinproduced a significant cytotoxicity on regulating the cell viability (Fig. 1). In the treatment of 1.25 μg/ml pyrethroids, permethrin promoted cellular growth of Sf-9, HEK293, and HeLa cells, β-cypermethrin facilitated the growth of Tn5B1-4 and HEK293 cells, and bifenthrin increased the growth of Tn5B1-4 cells. These cells possessed higher viability than the control ($P < 0.05$). However, as the used concentrations were increased, pyrethroids showed an increased trend to inhibit cell viability. In the treatment of 20 μg/ml pyrethroid, permethrin reduced the viability of the three insect cells and human HepG2 cells, β-cypermethrin decreased the viability of Sf-9 and S2 cells, and bifenthrin lowered the viability of S2 cells. These cells owned the viability less than 70% of the control. Moreover, the three pyrethroids showed stronger cytotoxicity on insect cells than human cells, but permethrin also inhibited the viability of HepG2 cells in a dose-dependent manner.

3.2. The cytotoxicity of neonicotinoids on cells

The cytotoxicity of neonicotinoids on cell viability was shown in Fig. 2. For insect cells, all treatments decreased the cell viability except for the increased growth of S2 cells in the treatment of 1.25 μg/ml nitenpyram. As the concentrations were increased, nitenpyram showed a stronger cytotoxicity on depressing the viability of Tn5B1-4 and Sf-9 cells, and acetamiprid exerted a higher inhibition on the viability of S2 cells ($P < 0.05$). The three neonicotinoids, however, exhibited no

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