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Insecticide cytotoxicology in China: Current status and challenges

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ABSTRACT

The insecticide cytotoxicology, as a new branch of toxicology, has rapidly developed in China. During the past twenty years, thousands of investigations have sprung up to evaluate the damages and clarify the mechanisms of insecticidal chemical substances to insect cells *in vivo* or *in vitro*. The mechanisms of necrosis, apoptosis or autophagy induced by synthetic or biogenic pesticides and virus infections have been systematically illuminated in many important models, including S2, BmN, SL-1, Sf21 and Sf9 cell lines. In addition, a variety of methods have also been applied to examine the effects of insecticides and elaborate the modes of action. As a result, many vital factors and pathways, such as cytochrome c, the Bcl-2 family and caspases, in mitochondrial signaling pathways, intracellular free calcium and lysosome signal pathways have been illuminated and drawn much attention. Benefiting from the application of insecticide cytotoxicology, natural products purifications, biological activities assessments of synthetic compounds and high throughput screening models have been accelerated in China. However, many questions remained, and there exist great challenges, especially in theory system, evaluation criterion, evaluation model, relationship between activity *in vitro* and effectiveness *in vivo*, and the toxicological mechanism. Fortunately, the generation of "omics" could bring opportunities for the development of insecticide cytotoxicology.

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1. Introduction

Developments in modern life technologies, such as cell and molecular biology, facilitate deep examinations into the body, focusing on cells, to analyze the effects of insecticides on insects. Insecticide cytotoxicology, a new branch derived from insect toxicology and cytotoxicology, aims to evaluate the damage and clarify the mechanism of insecticidal chemical substances to insect cells *in vivo* and *in vitro*. The concept generation, theory formation and application of insecticide cytotoxicology could not only provide new toxicology theories but also bring new methods and tools for the purifications of natural products, biological activities assessments of synthetic compounds, high-throughput screening models, molecular mechanisms illuminations of insecticides, and other related field. As a new subject, insecticide cytotoxicology deeply attract attention and interest of many pesticides scientists and insect toxicologists. Thus, a growing number of researchers engaged in this field, and obtained many results and important progresses. Here, we reviewed the current status and challenges of insecticide cytotoxicology based on evaluation models, cell death modalities, signal pathways and practical applications in China, which would provide novel insight into insecticide researches and developments.

2. Evaluation models

Establishment of a stable and comparable standardized evaluation model is the first step to carry out the toxicological researches. Various insect cultured cell lines have been used as models of insecticide cytotoxicology.

The fruit fly *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) has been widely used in genetics and developmental biology. As a common model organism, the cultured cell line S2 derived from *D. melanogaster* also played a vital role in insecticide development [1]. S2 cells [2,3,4] and the baculovirus-S2 system [5,6] had been widely used as eukaryotic expression vectors for molecular mechanism researches. Because *Bombyx mori* L. (Lepidoptera: Bombycidae) was designated as the model organism of Lepidoptera, BmN cell line and hemocytes derived from silkworm have become important models in the study of mechanisms of apoptosis and resistance [7,8,9,10,11]. Some vital models derived from Noctuidae, the largest family of Lepidoptera, including Sf21 [12] and Sf9 cells [13,14,15,16], derived from *Spodoptera frugiperda* (J.E. Smith), and SL-1 cells [17,18,19,20,21], derived from the *Spodoptera litura* Fabricius, had become the basic materials to study pesticide effects and programmed cell death. In addition, the TN-5B1-4 cell line [22,23], also known as Hi-5, derived from *Trichoplusis ni* Hübner, the Hz cell line [24,25] from *Heliothis Zea* Boddie, and the *Spex* cell line [12,26] from *Spodoptera exigua* Hübner were all important models to clarify the mechanism of a variety of insecticidal substances. With the development of the techniques for the isolation and culture of insect cells, there might be more evaluation models from specific organisms for particular mechanisms.

3. Cell death modalities

The active insecticidal substances could damage the metabolism of normal cells, eventually leading to cell death. The insecticidal efficacies of most pesticides were largely dependent on dose and time, but the forms of cell death, including necrosis, apoptosis, and autophagy, differed according to the stimuli. These death forms could be categorized according to differences in appearance, and complex cross talks between these mechanisms reported in relative medicines or diseases [27,28,29]. However, there are few studies focusing the mechanisms of insecticides on cellular level both at home and abroad.

3.1. Necrosis

Necrosis is a common phenomenon of living injured cells and is a feedback to external stimuli, disease, trauma, infection or other physical and chemical factors, which could result in the death of cells in living tissues through autolysis. The irreversible progression of necrosis included the dense clumping and progressive disruption of genetic materials and disruption of cell and organelle membranes [30,31]. For example, the cultured Sf9 cells showed necrosis symptoms after treatment at 55 °C for 30 min, including cell swelling and cell and nuclear membranes with fuzzy boundaries [13]. However, there have been several unidentified theories proposed and unsettling definitions, limiting understanding in necrosis. The new theory of programmed necrosis, or type III programmed cell death (PCD) [31,32], involving in cell swelling, organelle dysfunction and cell lysis, might largely accelerate this investigation progress.

Many insecticides could result in insect cell necrosis. Methomyl, a carbamate insecticide, could induce genotoxic effects by damaging DNA, including micronuclei, chromosome aberrations and sister-chromatid exchanges in S2 cells [33]. The sublethal concentrations of chlorpyrifos could induce significant concentration-dependent increases in single-strand DNA breaks and DNA fragmentation [34], triggering the controversy that organophosphate insecticides are potential genotoxic agents or not. Another effective insecticide against sucking pests, neonicotinoid, could also lead to the DNA damage of non-target organisms, attracting a lot of attentions [35]. Imidacloprid would lead to oxidative stress and DNA damage when exposure to earthworm [36,37] and zebrafish [38]. As an important and widely used biological pesticide, abamectin could not lead to DNA damage in hemocytes when the 4th instar newly exuviated silkworm larvae were fed with mulberry leaves treated with 1, 2, 4 μg/L avermectin after 96 h, but some morphological abnormalities were observed, including vacuole, deformation, swollen and condensed cytoplasm. However, when the insect was treated with a higher dose, 8 μg/L, avermectin could cause serious effects on DNA integrity [39,40].

Recent studies from diverse organisms showed that necrosis, which was considered to be an accidental or uncontrolled type of cell death, followed by a stereotypical series of cellular and molecular events. The entire procedure might suffer from organelles swelling, increased reactive oxygen species and cytoplasmic calcium, decreased ATP [41], activation of calpain and cathepsin protease, and rupture of organelles and the plasma membrane [31,42,43]. Although these researches provide evidences for conserved mechanisms of necrosis, but many details remain elusive.

3.2. Apoptosis

Apoptosis is a naturally occurring PCD mechanism characterized by depolymerization of cytoskeleton, cell shrinkage, chromatin condensation, nuclear fragmentation and translocation of phosphatidylserine to the cell surface. Apoptosis arises from a number of stimuli that initiate complex signaling pathways which lead to caspase cascade activation and cell death regulated by multiple specific genes and proteins [44,45]. Apoptosis prevents damaged cells from consuming essential nutrients and spreading infection to maintain stable internal environment. Since Kerr et al.(1972) first proposed the concept of apoptosis [46], this process now has been extensively studied in various fields to confirm its important roles in the germination, development, aging and degeneration of certain cells and tissues [28,29], and will provide molecular targets for new biorational insecticides innovation.

Apoptosis has become a hot topic in the fields of insecticide cytotoxicology in China. Methomyl [33,47], chlorpyrifos [34], phoxim [11], fipronil [4,48], imidacloprid [49], and many other insecticidal active products and/or chemicals, had been reported to induce apoptosis in concentration- and/or time-dependent manner in many cell lines. One of the most prominent researches is the apoptosis induced by botanical