Pesticide Biochemistry and Physiology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

Evaluation of the mechanism of anticancer activity of deltamethrin in Jurkat-J6 cell line

Neelima Sharma*, Sayantan Banerjee, Papiya Mitra Mazumder

Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra, Ranchi, India

ARTICLE INFO

Keywords:
Deltamethrin
Anticancer
Apoptosis
Caspase-3
Oxidative stress

ABSTRACT

Deltamethrin (DLM) is an α-cyano type-II synthetic pyrethroid compound which is extensively used in different agricultural and home pest control. The advantages of pyrethroids over other insecticides are that they are relatively non-toxic to birds and mammals and show high efficacy at relatively lower concentrations. DLM may have dual characteristics i.e. at low molar-concentration, it is nontoxic to normal healthy cells but can induce apoptotic cell death in cancerous cells. There is no reported work based on such hypothesis. Thus, this study has been designed to explore the anticancer property of DLM and the mechanism behind the apoptotic cell death by DLM in cancer cell line (Jurkat J6). Molecular docking study indicates that DLM has the greater binding affinity towards MCL-1 receptor. MTT assay has revealed some significant loss in the viability of cancerous cells by DLM. Further estimation of ROS and GSH have shown the significant oxidative stress induced by DLM in concentration-time dependent manner. DLM has also increased the caspase-3 activity and the apoptotic cells significantly while a decrease in interleukin-2 level has also been observed. The pre-treatment with thiol antioxidant and caspase inhibitor has confirmed the role of oxidative stress and the possibility of other pathways. These observations reveal that DLM may act as anticancer agent at lower concentrations (0.1–1 μM), though the further detailed investigation is warranted.

1. Introduction

Deltamethrin (DLM) is an α-cyano type-II synthetic pyrethroid insecticide which is extensively used in different agricultural and home pest control to eradicate the invasion of mites, ants, weevils and beetles. It is a synthetic insecticide structurally based on the natural pyrethrins, a chief chemical constituent of Chrysanthemum cineraraefolum (Lawrence & Casida, 1982). The pyrethroids are relatively non-toxic to birds and mammals having high efficacy at relatively lower concentrations. DLM is considered to be the safest class of available insecticides with relatively low mammalian toxicity due to the presence of ester group in the molecule (Bradburry & Coast, 1989). DLM containing products are widely available in the market and easy to purchase in everyday shops. They are applied in homes and gardens in form of aerosol fly sprays, pest strips, wood preservatives, to kill cockroaches, rodenticides, in shampoo to treat head lice's.

The emerging reports indicate that DLM induces apoptotic cell death mediated by oxidative stress and caspase-dependent pathways in various cells (Wu et al., 2003; Muhammad et al., 2011; Yu et al., 2014; Liu et al., 2015). DLM induces apoptosis in murine thymocytes and splenocytes within 25 μM–100 μM concentration range (Kumar et al., 2013; Kumar et al., 2016). It also decreases the calcium levels and thus induces the apoptosis in different cells like neural cells (Li et al., 2002).

Apoptotic cell death plays an important role in the mechanism of anticancer molecules which is controlled by a number of gene families. Literature reveals that the drugs targeting apoptotic pathways may contribute towards a promising anticancer therapeutic approach. Apoptosis induced by stimulating the extrinsic pathway can overcome the resistance to those therapeutic agents that act by causing damage in DNA. Targeting of the apoptogenic signaling pathways specifically in tumors is something of a holy grail for oncology. Regarding the anticancer activity of DLM, very few reports are available. Aydin (Aydin, 2011) observed the decreased antioxidant enzymes levels in lymphoid organs by thiacloprid, DLM and their combination. Tsai (Tsai, 2006) also proposed the use of the DLM as an antidepressant agent due to its inducible effect on brain-derived neurotrophic factor (BDNF) gene expression. In human glioblastoma cells, DLM has shown a significant apoptotic cell death (Hsu et al., 2012). It induced Ca^{2+} dependent apoptotic cell death in human oral cancer cell line at lower concentration (Chi et al., 2014).

Generally, humans are exposed to DLM at low concentrations, e.g. air concentration of DLM in spray, usage of DLM impregnated bed nets

* Corresponding author at: Department of Pharmaceutical Sciences and Technology, Birla Institute of Technology, Mesra, Ranchi 835215, India.
E-mail address: nsharmabit@gmail.com (N. Sharma).

https://doi.org/10.1016/j.pestbp.2018.06.010
Received 31 July 2017; Received in revised form 1 May 2018; Accepted 13 June 2018

and consumption of DLM contaminated food products, is likely to be less than the doses that are tested in the laboratories. Kumar et al. have observed that DLM is non-toxic to the murine thymocytes below 1 μM concentration (Kumar et al., 2013). Thus, it can be concluded that DLM may have dual characteristics, at lower concentration it may not cause apoptosis in normal healthy cells but can induce apoptotic cell death in cancerous cells. The hypothesis for the anticancer activity of DLM was proposed by Kumar et al. (Kumar et al., 2015a). As no reported work on DLM as an anticancer agent and its proper mechanism is available, therefore the present investigation has been aimed to explore the anticancer property of DLM and the probable mechanism underlying the apoptotic cell death in cancer cells. The use of insecticide as a potential anticancer agent could be strange but there are many medical agents whose medical application is far from their original use.

2. Materials and methods

2.1. Chemicals

DLM, an α-cyano type 2 synthetic pyrethroid ($C_{22}H_{19}Br_2NO_3$) was procured from Sigma-Aldrich having 98% purity. Jurkat-J6 cell line (70–80% confluent) was procured from National Centre for Cell Sciences, Pune. All chemicals used in this study were of highest grade purity available. 3(4, 5-dimethyl-2-yl) 2, 5 diphenyl tetrazolium bromide (MTT), DEVD-AFC substrate, O-phthaldialdehyde (OPT), dithiothreitol (DTT), 2, 7 dichlorofluorescein diacetate (DCFH DA) were purchased from Sigma-Aldrich (St. Louis, MO). Fetal bovine serum (FBS), trichloroacetic acid (TCA), triton-×100, dithiothreitol (DTT), RPMI-1640 media, antibiotic-antimycotic solution were purchased from Himedia Laboratories Ltd. (Mumbai, India).

2.2. Receptor and ligand

3D crystal structure of “EGFR-tyrosine kinase” (PDB ID: 1M17) and “myeloid cell leukemia” (PDB ID: 5FC4) were downloaded from protein data bank (PDB). Before docking, all the PDB structures were refined by removing associated water molecule and by adding polar H-bonds by using autodock tools. 3D structure of DLM was drawn by using ChemDraw Ultra 8.0 and energy was minimized (Kumar et al., 2015b).

2.3. Molecular docking

Molecular docking of DLM was performed in Maestro 9.3 (Schrodinger LLC Suite) with EGFR-tyrosine kinase receptor (PDB ID: 1M17) and myeloid cell leukemia receptor (PDB ID: 5FC4). Docking score of DLM and the respective internal ligand of those receptors were compared.

2.4. Cell culture

Jurkat-J6 cell line was procured from National Centre for Cell Sciences (Pune, India), having confluency > 80%. It is an immortalized line of human T-lymphocyte cells derived from acute T-cell leukemia. It is used to study acute T-cell leukemia. Jurkat-J6 cell line has the ability to produce IL-2 and that's why it is useful for different anti-cancer study. The procured cells were centrifuged, resuspended in complete RPMI-1640 media (containing 10% FBS and 1% antibiotic-antimycotic solution) in T-25 suspension-cell culture flask (Himedia Laboratories, Mumbai) and incubated at 37 °C with continuous 5% CO_2 supply. Cell density was adjusted to about 1.5×10^6 cells/mL for all the further experiments (Curnock et al., 2001).

2.5. Cytotoxicity assay

MTT assay was carried out to determine the viability of Jurkat Cells. The cells at a concentration of 1.0×10^4 cells were seeded in 96-well plate. Different concentrations of DLM (0.01, 0.1, 0.5, 1, 10, 50 and 100 μM) were added and incubated for 18 h at 37 °C with 5% CO_2. 4 h prior to the completion of incubation, 10 μL MTT (5 mg/mL) was added to each well. After the incubation, the plate was centrifuged, the supernatant was discarded and 100 μL DMSO was added to each well. The absorbance was taken at 570 nm in a microtiter plate reader (Thermo Scientific). According to result of MTT assay, 3 concentrations of DLM (0.1, 0.5 and 1 μM) were selected for the further assays (Mosmann, 1983).

Table 1
Docking studies of DLM with 1 M17 and 5FC4 receptors.

PDB ID	Name of compound	Docking score	Polar H-bond	π-π interactions
1M17	Internal Ligand	-8.58	Asp 831 Cys 773 Met 769	Val 702
	Deltamethrin	−3.48	Met 769	Val 702
5FC4	Internal Ligand	−8.07	Arg 263 (2)	Phe 270 Phe 228
	Deltamethrin	−6.73	No such interaction observed	Val 253 Leu 267

2.6. Reactive oxygen species (ROS) measurement

Jurkat-J6 cells were adjusted to 1.5×10^6 cells/mL and 200 μL cell suspension was added to each well. Different concentrations of DLM (0.1, 0.5 and 1 μM) and 2 μL DCFH DA (100 μM final concentration) were added simultaneously and cells were incubated for 30 min, 1 h, 3 h and 6 h. After incubation cells were resuspended in PBS and resultant fluorescence was measured at excitation and emission wavelength of 480 nm and 530 nm respectively (Wu & Yotnda, 2011).

2.7. Glutathione measurement

The cells treated with different DLM concentrations (0.1, 0.5 and 1 μM) for 30 min, 1 h, 3 h and 6 h were resuspended in 100 μL phosphate-EDTA buffer and sonicated for 10 min. 100 μL TCA was further added and incubated on ice for 10 min. After the incubation, cells were centrifuged and the supernatant was collected. 10 μL (1 mg/mL) O-phthaldialdehyde was added and resultant fluorescence intensity was measured at excitation and an emission wavelength of 390 nm and 470 nm respectively (Kumar et al., 2015c).

2.8. Caspase-3 activity measurement

A population of 3.0×10^6 cells/ml was incubated with DLM (0.1, 0.5 and 1 μM) for 1.5 h, 3 h and 6 h. After incubation, cells were centrifuged, resuspended in 50 μL lysis buffer and incubated for 10 min on ice. The supernatant was collected and 50 μL reaction buffer and 5 μL DEVD-AFC (50 μM final concentration) were added and further incubated for 2 h at 37 °C. The resultant fluorescence was measured by using excitation and an emission wavelength of 400 nm and 505 nm respectively (Kumar et al., 2015c).

2.9. Interleukin-2 measurement

The Jurkat cells, incubated with DLM (0.1, 05 and 1 μM) for 1.5 h, 3 h and 6 h at 37 °C, were centrifuged and the supernatant was collected. 100 μL of the supernatant was added to each well of the pretreated 96-well plate. The plate was sealed and incubated for 2 h at 37 °C. The plate was washed with wash buffer, 100 μL detection antibody was added and incubated at 37 °C for 2 h. 100 μL diluted Streptavidin-HRP (Horseradish Peroxidase) was added after the washing and further incubated at 37 °C for 30 min. 100 μL freshly