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Chemosphere 66 (2007) 474–479

CHEMOSPHERE

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Antiandrogenic activity of pyrethroid pesticides and their metabolite in reporter gene assay

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Received 16 March 2006; received in revised form 29 May 2006; accepted 31 May 2006
Available online 20 July 2006

Abstract

Many pesticides possess hormonal activity and have thus been classified as endocrine disruptors. Pyrethroids are commonly used pesticides worldwide, but little has been done to characterize their antiandrogenic activity potential. We tested three frequently encountered pyrethroids (fenvalerate, cypermethrin, permethrin) and their metabolite 3-phenoxybenzoic acid (3-PBA) for antiandrogenic and androgenic activity using a human androgen receptor (AR) mediated luciferase reporter gene assay in CV-1 African green monkey kidney cell. The assay displayed appropriate response to the known AR agonist 5α-dihydrotestosterone and AR antagonist nilutamide and flutamide. At 0.1 mM, all the three tested pyrethroids significantly suppressed the luciferase expression. Further, their metabolite 3-PBA also showed antagonist activity. None of the test chemicals showed androgenic activity. Through the antiandrogenic pathways, exposure to certain pyrethroids may contribute to the damage of reproductive system. In conclusion, pyrethroid pesticides can act as antiandrogen *in vitro*, and metabolizing to 3-PBA cannot eliminate the antagonist activity. This result provides useful information for risk assessment of pyrethroid pesticides.

Keywords: Androgen receptor; Endocrine disruptor; Fenvalerate; Cypermethrin; Permethrin; 3-Phenoxybenzoic acid

1. Introduction

In recent years, public and researchers have expressed increasing concern that pesticides and many other environmental chemicals have hormonal activity, and thus modify the normal functioning of human and wildlife endocrine systems (Colborn et al., 1993; Colborn, 1995). A major mechanism of endocrine disruption is the action of chemicals as receptor agonists or antagonists through direct interaction with hormone receptors, thus altering endocrine function. In particular, chemicals mimicking endogenous estrogen via estrogen receptor (ER) have been the focus of research for the last 20 years (Kojima et al., 2004). Meanwhile, recent studies have shown that several chemicals may exert antiandrogenic effect by interfering with androgen receptor (AR) (Sohoni and Sumpter, 1998; Vinggaard et al., 1999). Some chemicals including the bioaccumulating DDT metabolite *p*,*p*′-DDE (dichlorodiphenyldichloroethylene), bisphenol A, octylphenol and nonylphenol have demonstrated AR-mediated antiandrogenic activities *in vitro* (Gray et al., 1999; Xu et al., 2005). However, we know comparatively little about the interference of pesticides with human AR.

Pesticides are commonly used for the control of agricultural and indoor pests (Garey and Wolff, 1998). Synthetic pyrethroid pesticide is one of the most popular pesticides used in China. They are known to exert their pesticidal actions by altering the sodium permeabilities of insect nerve membranes by modulating voltage-sensitive sodium channels (Bloomquist, 1996). Laboratory evidence suggested that pyrethroids were relatively safe to humans

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0045-6535/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2006.05.059

and wildlife (Miyamoto et al., 1995). However, Colborn et al. (1993) have already included pyrethroids in their lists of possible endocrine disruptor chemicals (EDCs). Recently, several *in vitro* studies have evaluated the potential hormonal activity of pyrethroids (Eil and Nisula, 1990; Miyamoto et al., 1995; Gaido et al., 1997; Saito et al., 2000). Most of these studies of pyrethroids are their ability to interact with estrogen receptors, yet little has been done to assess their interaction with human AR.

To study the interaction between chemicals and AR, we developed a transient reporter gene assay based on CV-1 African green monkey kidney cell. The receptor reporter gene assay was proposed by the United States EPA for inclusion in a Tier 1 screening battery (T1S) to detect EDCs acting as receptor agonists and antagonists (EDSTAC, 1998). Binding of androgen to AR in target cells results in the initiation of specific transcription activation events. Therefore, the introduction of artificial, AR-regulated reporter gene constructs into cells has become a useful method of measuring AR transcriptional activation. The present reporter assay utilized the human AR for transcriptional regulation of a luciferase reporter gene. The objective of the study was to evaluate the possible androgenic and antiandrogenic activity of pyrethroids and their metabolite.

In this study, we tested three most commonly used pyrethroids including fenvalerate, cypermethrin and permethrin which were suspected to possess endocrine disruptor activity. Fenvalerate and cypermethrin are both type II pyrethroid insecticides, meaning they have an α-cyano group at the α-carbon position of the alcohol moiety. Our previous study showed that fenvalerate could induce significant reduction in testis weight, epididymal sperm count, sperm motility and marker testicular enzymes for testosterone biosynthesis (Bian et al., 2004; Xia et al., 2004, 2005; Xu et al., 2004). Ingestion of cypermethrin at 18.93 and 39.66 mg d^{-1} resulted in a significant decrease in the perimeter and number of cell layers of the seminiferous tubules. Epididymal and testicular sperm counts as well as daily sperm production were significantly decreased in cypermethrin exposed males (Elbetieha et al., 2001). These results demonstrated the adverse effects of fenvalerate and cypermethrin on fertility and reproduction in male rats. And permethrin, a type I pyrethroid, also showed antiandrogenic activity in Hershberger assay (Kim et al., 2005). In the present study, we detected significant antiandrogenic activity of the three pesticides and their major metabolite 3-phenoxybenzoic acid (3-PBA) in the AR-mediated reporter gene assay.

2. Materials and methods

2.1. Chemicals

Test chemicals were the highest grade available for the environmental analysis. The source, purity, CAS and abbreviation of chemical were listed in Table 1. Cypermethrin and permethrin were obtained from Dr. Haiyan Chen (Nanjing Medical University, Nanjing, China). Dexamethasone and 5α-dihydrotestosterone (5α-DHT, purity >99%) were purchased from Sigma Chemical CO. (St. Louis, MO, USA). Chemical structures of the pyrethroid pesticides tested in this study were shown in Fig. 1. Stock solutions of the chemicals were prepared in absolute ethanol at a concentration of 10^{-1} M, stored at −20 °C, and diluted to desired concentrations in phenol red-free RPMI1640 medium (Sigma Chemical CO.) immediately before use. The final ethanol concentrations in the culture medium did not exceed 0.2% (v/v) that did not affect cell yields.

2.2. Plasmids and cell line

We constructed a reporter plasmid pMMTV-LUC based on the pGl3-Basic vector (Promega, Madison, WI, USA). The oligonucleotides of mouse mammary tumor virus (MMTV) containing four androgen response element (ARE) sequences and TATA promoter were synthesized and inserted into the *Kpn* I and *Bgl* II sites of pGl3-Basic

Table 1
Data on test chemicals

Chemicals and abbreviation	Supplier	CAS	Purity (%)
Cypermethrin	Gift	52315-07-8	>99
Flutamide	Sigma	13311-84-7	>99
Nilutamide	Sigma	63612-50-0	>98
Permethrin	Gift	52645-53-1	>99
3-Phenoxybenzoic acid (3-PBA)	Accustandard[a]	3739-38-6	>99

[a] Accu Standard Chemicals (New Haven, CT, USA).

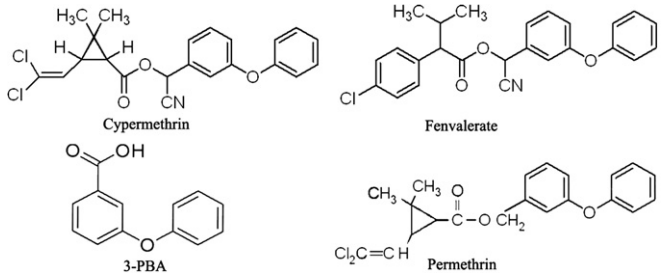

Fig. 1. Structures of three synthetic pyrethroid pesticides and their metabolite 3-PBA.