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Original research article

The 28-day exposure to fenpropathrin decreases locomotor activity and reduces activity of antioxidant enzymes in mice brains☆

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ABSTRACT

Background: Fenpropathrin (Fen) is a pyrethroid (Pyr) insecticide. Pyrs are used in veterinary medicine, in agriculture and for domestic purposes. As their use increases, new questions about their side effects and mode of action in non-target organisms arise.

The objective of this work was to characterize dose–response relationship for *in vivo* motor function and memory in mice exposed to Fen for 28 days and to assess its influence on activity of antioxidant enzymes in mice brains.

Methods: The experiment was performed using 64 female mice. Fen at the dose of 11.9 mg/kg of body mass, 5.95 mg/kg or 2.38 mg/kg was administered *ip* to the mice for 28 consecutive days. Motor function and spatial working memory were tested on days 7, 14 and 28. On day 29, the animals were sacrificed and brains were used to determine activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx).

Results: Fen significantly decreased locomotor activity in mice receiving the highest dose at every stage of the experiment. Lower doses reduced locomotion on days 7 and 14. Fen did not produce memory impairment. A decrease in activities of SOD and GPx was recorded in mice brains.

Conclusions: The decrease of SOD activity in mice brains results from direct inhibition of the enzyme by Fen and/or increased utilization due to excessive free radical formation in conditions of Fen-induced oxidative stress. The reduction in GPx activity is probably due to limited glutathione availability. The reduced locomotor activity is a behavioral demonstration of Fen-induced damage in the dopaminergic system.

Introduction

Fenpropathrin (Fen) is a pyrethroid (Pyr) insecticide. Pyrs are used in veterinary medicine against broad range of ectoparasites in large and small animals, for crop protection in agriculture as well as for multiple domestic purposes (to control mosquitoes, flies, ants, and cockroaches) [1]. As their use increases, new questions about their side effects and mode of action in non-target organisms arise.

In 1980, Verschoyle and Aldridge [2] characterized acute toxicity of 36 Pyrs following their intravenous administration. They established the first taxonomy of Pyrs' intoxication in mammals. They divided the chemicals into the ones producing aggressive sparring, sensitivity to external stimuli, tremor progressing to prostration (T syndrome or Type I) and choreoathetosis, salivation, pawing, burrowing behavior, increased startle response and terminal clonic seizures (CS syndrome or type II) [2]. This classification was confirmed in studies of the intracerebral toxicity of 29 Pyrs in mice [3]. The main difference in the results of these studies was that Fen, classified as producing T syndrome in the first study, was classified as producing both syndromes of intoxication (tremor and salivation) in the second study.

In recent studies, Pyrs were shown to produce imbalance between oxidants and antioxidants, or oxidative stress (OS) [4]. The OS is considered one of the possible mechanisms of neurotoxic effects of Pyrs in mammals [5,6].

Fen is an α-cyano pyrethroid (α-cyano-3-phenoxybenzyl-2,2,3,3-tetramethylcyclopropane carboxylate) widely used for pest control [7]. Majority of Pyrs having a structure of esters of α-cyano-3-phenoxybenzyl alcohols produce the CS syndrome in mammals like deltamethrin does [8]. However, Fen is not a typical α-cyano Pyr.

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Pyrs are neurotoxins acting primarily on the voltage-sensitive sodium channels in excitable cells (neurons and muscles). The altered sodium channels cause repetitive firing and depolarizing block in neurons [9].

In insects, which are target organisms for Pyrs, acute intoxication produces reversible impairment of motor function that may be followed by death. Moderate doses of Pyrs produce hyperexcitability, an increase in motor activity with altered mode of flying or walking [10]. Higher doses cause immobility of walking insects and falling down among flying insects [11,12]. The highest doses cause immediate hindlimb paralysis, movement incoordination and eventually prostration and death [13]. Sublethal effects (movement incoordination, hyperactivity, disorganization of calling behavior and chemical communication systems followed by disruption of sexual behaviors) may occur in insects after exposure to 1/10 of an insect LD_{50} dose. Honey bees and other pollinators being non-target organisms may also fall victim to Fen and other pesticides used in agriculture.

When administered in high doses Pyrs produce neurotoxicity in non-target organisms, including mammals. According to veterinary observations, acute, sublethal exposure to Pyrs produces restlessness, hyperexcitability followed by locomotive ataxia (“drunken movements”), mydriasis, diarrhea and general depression, motor incoordination, paresis, head hobbing, chewing, hypersalivation and whole body tremors [12].

Exposure of sheep for 6 weeks to supermethrin administered orally decreases weight gain, produces nervous irritability, head and neck tremors, increases vestibular and palpatory sensitivity and causes conjunctivitis, lacrimation, mucous discharge, weakness of the hind legs, diarrhea, uncoordinated movements, ataxia, convulsions and death. The clinical manifestation of supermethrin poisoning resembles cyanide poisoning because the α-cyano moiety in Type II Pyrs is released by rumen bacteria in ruminants [14]. Subchronic intoxication with a Pyr sumi-alpha leads to significant disorders in immune reactivity in rats: pronounced changes of macrophagocytic function, reduction of cell-mediated and humoral immunity and accumulation of pathogenic circulating immune complexes [15].

Pyrs are considered to be relatively safe for mammals because of their constant, and higher than in insects, internal body temperature, faster metabolism and lower sensitivity of sodium channels [9]. However, humans may be exposed to the insecticides at workplace (especially farmers and greenhouse workers) [16] and there were numerous cases of Pyr poisoning in non-target organisms described [1]. Moreover, people and animals may be exposed to traces of Pyrs in food and water [17–20] as well as due to their long-term presence in the indoor air and textiles [21,22]. Therefore a question arises whether Pyrs act in a dose-additive fashion and if interaction with sodium channels is the only mechanism of their action.

The objective of this work was to characterize dose–response relationship for *in vivo* motor function and memory in mice exposed to Fen for 28 days and to assess its’ influence on activity of antioxidant enzymes in mice brains for better understanding of possible effects of Pyrs in mammals, especially humans.

Materials and methods

All the experimental procedures were conducted with respect to the law regulations of the European Community and Poland. They were conducted at the Chair and Department of Hygiene, Medical University of Lublin, Poland. The Local Ethics Committee for Animal Experiments in Lublin had approved the experiment (Opinion No. 4/2009, dated: Jan. 9th 2009). The experiments were performed between 8:00 a.m. and 6:00 p.m.

Animals

Non-gravid female albino Swiss mice weighing 18–24 g approximately 6 weeks of age purchased from a licensed breeder (T. Górzkowski, Warszawa, Poland) were used in the study. All animals were given a 7-day acclimation period and maintained on a 12 h light/dark cycle. Food and tap water were provided *ad libitum*. Temperature was maintained at 21 ± 2 °C. Sixty-four animals were used in the experiment.

Chemicals

Fen (RS)-α-cyjano-3-phenoxybenzyl 2,2,3,3-tetramethylcyclopropane carboxylate was purchased from the manufacturer (Institute of Industrial Organic Chemistry, Annopol, Warszawa, Poland). As pyrethroids poorly dissolve in water, Tween 60 (polyoxyethylene sorbitan monostearate) purchased from Laboratorium Reagenzien, Frankfurt, Germany was used to prepare solutions in saline (0.1 ml of Tween/9.9 ml of saline).

Drug treatment

The LD_{50} for Fen in mice was calculated in our laboratory with Lichtfield and Wicoxon’s method to be 23.8 mg/kg of body mass [23]. In this work, we aimed to characterize dose–response relationship for *in vivo* effects of subacute poisoning with Fen; therefore, the doses of 0.5 LD_{50} = 11.9, 0.25 LD_{50} = 5.95 and 0.1 LD_{50} = 2.38 mg/kg were chosen. The same doses were already used in another study of ours on subacute poisoning with Fen in mice [24]. The dermal and oral routes of exposures to Pyrs are most often reported in publications about poisoning of non-target organisms (humans, cats and dogs) [1,16]. Dermal application requires shaving and gavage is stressful for animals. The intracerebral [3] or intravenous dosing [2], used in animal experimental toxicity models, has little relevance to the risk assessment in ecotoxicology. Therefore, the intraperitoneal route of administration was chosen. It is a fast and easy mode of drug application producing little pain to the animals. The rich web of blood vessels and large surface of the peritoneum guarantee good absorption of the xenobiotic.

To prepare a solution of 11.9 mg Fen/kg of mice body mass, a dose of 11.9 mg of Fen was dissolved in 9.9 ml of saline with 0.1 ml of Tween. To make solutions of Fen at the dose of 5.95 mg/kg or 2.38 mg/kg, respective amounts of Fen, saline and Tween were used. A mouse weighing 20 g received 0.2 ml of an appropriate solution per injection. A volume of 10 ml solution was prepared per 1 kg of mice body mass. Mice were randomly divided into eight groups of eight mice:

I. Control-receiving saline
II. Control-receiving saline
III. Receiving Fen at the dose of 2.38 mg/kg of body mass
IV. Receiving Fen at the dose of 2.38 mg/kg of body mass
V. Receiving Fen at the dose of 5.95 mg/kg of body mass
VI. Receiving Fen at the dose of 5.95 mg/kg of body mass
VII. Receiving Fen at the dose of 11.9 mg/kg of body mass
VIII. Receiving Fen at the dose of 11.9 mg/kg of body mass

Experimental design

Animals were daily injected intraperitoneally (*ip*) with saline or respective dose of Fen for 28 consecutive days. Groups I, III, V and VII were tested in Opto-Varimex 4 Activity Meter (Columbus, OH, USA) for evaluation of locomotor activity. Each monitoring instrument (Opto-Varimex) consisted of a Plexiglass cage (44.5 cm × 44.5 cm)