Medycyna Środowiskowa – Environmental Medicine 2022, Tom 25, Nr 3–4, 72–76
www.environmed.pl

ORIGINAL ARTICLE

Assessment of neurobehavioural effects of exposure to Fury 100 EW (zeta-cypermethrin) pyrethroid and caffeine in mice

Ocena neurobehawioralnych skutków ekspozycji na pyretroid Fury 100 EW (zeta-cypermetrynę) i kofeinę u myszy

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Łukawski K, Zygan-Filipiak K. Assessment of neurobehavioural effects of exposure to Fury 100 EW (zeta-cypermethrin) pyrethroid and caffeine in mice. Med Srod. 2022; 25(3–4): 72–76. doi: 10.26444/ms/162164

Abstract

Introduction and Objective. Pyrethroids are widely used pesticides in agriculture. Growing evidence suggests that they may be harmful for human health. Experimental studies show that pyrethroids can induce seizures and impairments of cognitive and motor functions in animals. Caffeine, a widely used psychoactive substance, may influence these disorders. The aim of the current study was to assess whether co-exposure to pyrethroid (zeta-cypermethrin) and caffeine could affect their neurotoxic potential.

Materials and method. The study was conducted in adult male Swiss mice. The effects of combined exposure to zeta-cypermethrin and caffeine on pyrethroid-induced convulsions, as well as learning in the passive avoidance test and motor coordination in the rota-rod test, were assessed in animals. In the current study, Fury 100 EW, an insecticide with zeta-cypermethrin as an active ingredient, was used. Substances were administered as single intraperitoneal injections. Caffeine was used at doses affecting the neurobehavioural effects of other pesticides, as previously described.

Results. Zeta-cypermethrin-induced seizures were not influenced by caffeine (40 mg/kg) administration. Pyrethroid, at its 1/5 CD_{50}, did not impair learning or motor coordination in mice. Caffeine did not interact with zeta-cypermethrin in the behavioural tests.

Conclusions. Based on the results obtained, it can be concluded that combined exposure to zeta-cypermethrin and caffeine has no effect on their neurotoxic potential in mice.

Key words

pyrethroids, caffeine, seizures, memory, motor coordination, mice

Streszczenie

Wprowadzenie i cel pracy. Pyretroidy są pestycydami szeroko stosowanymi w rolnictwie. Coraz więcej dowodów sugeruje, że mogą one być szkodliwe dla zdrowia ludzkiego. Badania eksperymentalne wykazały, że pyretroidy mogą wywoływać drgawki i upośledzenie funkcji poznawczych i motorycznych u zwierząt. Kofeina, szeroko stosowana substancja psychoaktywna, może wpływać na te zaburzenia. Celem niniejszego badania była ocena, czy jednoczesna ekspozycja na pyretroid (zeta-cypermetrynę) i kofeinę może wpływać na ich potencjał neurotoksyczny.

Materiał i metody. Badanie przeprowadzono na dorosłych samcach myszy Swiss. Oceniono wpływ łącznego narażenia na zeta-cypermetrynę i kofeinę na drgawki wywołane pyretroidem, a także na uczenie się w teście biernego unikania i koordynację ruchową w teście rota-rod u zwierząt. W obecnym badaniu zastosowano Fury 100 EW, insektycyd z zeta-cypermetryną jako substancją czynną. Substancje podawano w pojedynczych iniekcjach dootrzewnowych. Kofeinę stosowano w dawkach wpływających na efekty neurobehawioralne innych pestycydów.

Wyniki. Podanie kofeiny (w dawce 40 mg/kg) nie wpłynęło na drgawki wywołane zeta-cypermetryną. Pyretroid, w dawce 1/5 CD_{50}, nie zaburzył uczenia się ani koordynacji ruchowej u myszy. Kofeina nie wchodziła w interakcje z zeta-cypermetryną w testach behawioralnych.

Wnioski. Na podstawie uzyskanych wyników można stwierdzić, że łączna ekspozycja na zeta-cypermetrynę i kofeinę nie ma wpływu na ich potencjał neurotoksyczny u myszy.

Słowa kluczowe

pyretroidy, kofeina, drgawki, pamięć, koordynacja ruchowa, myszy

INTRODUCTION

Pyrethroid insecticides are widely used for agricultural and household purposes as well as for public health and commercial concerns, including mosquito control, Dengue

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viruses, malaria, and bed bug infestations [1]. The levels of pyrethroid residues in the environment across the world have significantly grown as a result of this increased usage [2]. Pyrethroids are thought to be generally safe for mammals, but there is growing evidence that they have adverse effects on human health after exposure to low environmental doses [3]. There are two major types of pyrethroids: Type I pyrethroids do not have a cyano moiety at the alpha position, whereas Type II pyrethroids do [4]. In mammals, high doses of Type II pyrethroids cause choreoathetosis and salivation (CS), or CS syndrome that includes writhing, salivation, pawing, burrowing, tremors, and clonic seizures [4]. Furthermore, experimental studies have shown that Type II pyrethroids can impair learning and motor functions in rodents [5, 6]. Cypermethrin and zeta-cypermethrin are examples of Type II chemical compounds. Cypermethrin is a Type II pyrethroid insecticide composed of eight isomers, each with three chiral centres while zeta-cypermethrin is enriched in four alpha-S enantiomers [7].

Zeta-cypermethrin [(S)-cyano-3-phenoxyphenyl-methyl-cis-trans-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane carboxylate], which was utilized in the current study, has high selectivity for insects and also acts on mammalian sodium channels, producing prolonged excitation and affecting central nervous system functions by increasing sodium membrane permeability and disrupting action potentials. Other biochemical targets of this pesticide include calcium channels and calcium-magnesium-ATPases [8].

Recently, it has been reported that the effects of pyrethroids on the central nervous system can be affected by caffeine (1,3,7-trimethylxanthine) [9]. Caffeine, a non-selective adenosine A_1 and A_{2A} receptor antagonist, is the most popular psychoactive substance ingested worldwide [10] and its use is increasing [11]. Among the most consumed beverages that contain caffeine are coffee and tea. Caffeine is also synthesized and added to meals, soft drinks, energy drinks and several medicines [12]. In Poland, average caffeine consumption among adults slightly exceeds the safe consumption dose established by the European Food Safety Authority [13]. The underlying motivations for increased caffeine uptake in the world, are mostly improved concentration and memory, as well as physical performance [11]. In fact, moderate CAF consumption is believed to increase or improve energy availability, alertness, wakefulness, the ability to concentrate and focus attention, cognitive functioning and motor performance, as well as reduce fatigue and the perception of effort during physical exercise [14]. Moreover, experimental studies have documented that caffeine intoxication at high doses, similarly to pyrethroids, can induce convulsions or cognitive deficits [15, 16, 17, 18]. On the other hand, caffeine administration may result in the enhancement of memory and motor coordination functions in animals [18]. However, this improving effect of caffeine has not yet been observed for its concomitant administration with pyrethroids. In contrast, caffeine has been demonstrated to potentiate the harmful impact of tefluthrin, a pyrethroid, on motor coordination in mice [9].

Similarly to caffeine-drug interactions (e.g., caffeine-antiepileptic drug interactions), caffeine-pyrethroid interactions can also occur and may have a negative impact on human health after exposure to caffeine and pyrethroid. Pyrethroids are pesticides that are recognized for their neurotoxicity, which can result in seizures and impaired cognitive and motor functions. Caffeine, a non-selective adenosine A_1 and A_{2A} receptor antagonist and widely used psychoactive drug, affected pesticides' activity in neurobehavioural tests (passive avoidance task, rota-rod test) as documented for chlorpyrifos [19] and tefluthrin [9].

OBJECTIVE

The aim of the study was to ascertain whether co-exposure to zeta-cypermethrin, a pyrethroid pesticide, and caffeine, influence their neurotoxic potential in the neurobehavioural tests and on seizure activity. It was hypothesized that caffeine may potentiate the neurotoxicity of zeta-cypermethrin, as it did in the case of previously tested pesticides [9, 19].

MATERIALS AND METHOD

Animals. The tests were performed on adult male Swiss mice weighing 25 – 30 g, purchased from a licensed dealer. The animals were kept in colony cages with free access to food and water, in standard laboratory conditions (room temperature 22 ± 2°C, relative humidity 55 ± 10%, 12-h light/dark cycle). After a period of adaptation to laboratory conditions, the animals were randomly divided into experimental groups of eight mice. Each animal was used in only one experiment. The tests were carried out from 09.00–16.00 in constant ambient conditions (temperature, lighting, noise level). The total number of mice used in this study was 128. Experiments were conducted in accordance to the ARRIVE guidelines. All experimental procedures were approved by the Second Local Ethics Committee at the University of Life ciences in Lublin, and complied with the EU Directive 2010/63/EU for animal experiments.

Substances. The following substances were used: zeta-cypermethrin (Fury 100 EW; FMC Chemical, Brussels, Belgium), and caffeine (coffeinum-natrium benzoicum) purchased from Pharma Cosmetic (Kraków, Poland). Fury 100 EW is a commercial insecticide with zeta-cypermethrin as an active ingredient. Insecticide and caffeine were dissolved in saline (0.9% NaCl). They were given intraperitoneally (i.p.) in a volume of 5 ml/kg body weight as single injections. Zeta-cypermethrin was administered 60 min and caffeine was injected 30 min before the tests. A recent work by the authors of the current study investigated the effect of caffeine at the dose of 40 mg/kg on the neurotoxicity of pyrethroids [9]. The dose of caffeine employed in the study was also 40 mg/kg for comparison purposes.

Seizure activity. Based on the findings reported by Weiner et al. [5], zeta-cypermethrin-induced seizure activity was evaluated. The study described pyrethroid-evoked clonic convulsions as the presence of at least one of the following conditions: repetitive mouth/jaw movements, back twitches, head/body twitches, irregular jerking, and generalized convulsions. Zeta-cypermethrin doses ranged from 10 – 100 mg/kg. Zeta-cypermethrin was given to four groups of mice, each with eight animals, in different doses, alone or in conjunction with caffeine (40 mg/kg). Following the administration, each animal was placed in a separate transparent Plexiglass cage (25 × 15 × 10 cm),

and the occurrence of clonic seizures was monitored for up to three hours. A dose-response relationship curve was calculated based on the percentage of animals having seizures to estimate the corresponding CD_{50} value (median pyrethroid convulsive dose generating a seizure response in 50% of mice) for pyrethroid alone, or in combination with caffeine. In subsequent behavioural experiments, a pyrethroid dose of 1/5 CD_{50} was used.

Passive avoidance test. To assess learning in mice, a step-through passive avoidance task was used. On the first day, during the training trial, the animals were pretreated with the insecticide and/or caffeine, and placed in an illuminated box (12 × 20 × 15 cm) adjacent to a dark box (24 × 20 × 15 cm). The dark box was equipped with an electric grid floor connected to a generator, and a 4 × 7 cm doorway was positioned in the centre of a shared wall between boxes at the floor level. The mouse was punished with an electric foot shock (0.6 mA for 2 s) when it entered the dark compartment. The next day, 24 h later, the same animals (without any treatment) were placed in the illuminated box, and the latency (retention time) of entering the dark box was recorded. The experiment terminated when the mouse entered the dark box or after 180 s. Animals that avoided the dark box for 180 s were thought to remember the task.

Rota-rod test. A rota-rod apparatus (model 47600, Ugo Basile, Varese, Italy) was used to evaluate motor coordination in mice. The animals were trained on a 3-cm diameter rod rotating at a constant speed of 6 rpm during the first day of the experiment. Mice that remained on the rod for at least 60 s in two consecutive trials (120 s) were tested further. Animals were pretreated with the insecticide and/or caffeine the next day, 24 h later, and they were then put back on the rotating rod for 120 s. The moment the animals fell off the rota-rod was recorded. Mice that did not fall off within the time limit received a score of 120 s.

Statistics. CD_{50} values and 95% confidence limits were calculated by the computer log-probit analysis according to Litchfield and Wilcoxon [20]. The log-probit method was employed to analyze the seizure activity of zeta-cypermethrin and caffeine. The results of the rota-rod test and the passive avoidance task were assessed using Kruskal-Wallis non-parametric ANOVA, and Dunn's multiple comparisons test. At $p < 0.05$, group differences were considered statistically significant.

RESULTS

Seizure activity. As seen in Figure 1, i.p. administration of zeta-cypermethrin induced seizure-like activity in mice (CD_{50} = 16.1 mg/kg). Treatment with caffeine at a dose of 40 mg/kg i.p. had no significant impact on the pyrethroid CD_{50} value (CD_{50} = 14.0 mg/kg).

Behavioural tests. Zeta-cypermethrin i.p. alone, applied at its 1/5 CD_{50} (3.2 mg/kg), did not impair learning in the passive avoidance task or motor coordination in the rota-rod test. Although caffeine (40 mg/kg i.p.) administered individually had no effect on learning and motor coordination in mice, it potentiated the neurotoxic effect of pyrethroid (tefluthrin) on motor balance in the rota-rod in the previous study [9]. However, this was not the case in the current study. Co-exposure to zeta-cypermethrin (3.2 mg/kg) and caffeine (40 mg/kg) did not cause either cognitive or motor coordination deficits, compared to the control group (Tab. 1). Biochemical measurements were not performed due to the negative behavioural test results.

DISCUSSION

The study demonstrates that acute exposure to zeta-cypermethrin (Fury 100 EW) can induce seizure-like activity in mice. This effect of zeta-cypermethrin intoxication was not modified by caffeine treatment. The combined administration of zeta-cypermethrin and caffeine did not cause learning and motor impairments in animals.

Cypermethrin (Type II pyrethroid) is toxic not just for insects but also for mammals, and symptoms/signs, such as muscle tremors, ataxia, limb weakness, convulsions, coma, and death from respiratory depression, have been documented in animals exposed to high doses of cypermethrin [21]. Cypermethrin induced severe tonic-clonic convulsions when administered centrally (intracerebroventricularly) in rats (50 µg/animal), as well as peripherally (i.p.), at the dose of 60 mg/kg in mice [22]. Repeated exposure to cypermethrin (300 mg/kg, i.p.) induced progressive development of epileptiform activity when the electroencephalographic (EEG) activity was recorded in rats. Some of the paroxysmal events were present with generalized tonic-clonic seizures [23].

The results of the current study demonstrate for the first time that zeta-cypermethrin, similarly to cypermethrin, is a proconvulsant agent in rodents. The mechanism by which zeta-cypermethrin induces seizure activity remains unknown. Pyrethroids' principal action is to disrupt the nerve membrane by delaying the closure, inactivation, or long-term opening of voltage-sensitive sodium channels (VSSC), resulting in additional sodium ions crossing the membrane and depolarizing the neural membrane beyond the normal extent [24].

Type II pyrethroids can modulate the action of GABA-gated chloride channels, voltage-dependent chloride channels, ATPase and voltage-gated calcium channels, calcium-dependent release of neurotransmitters and protein kinase C (PKC)-dependent protein phosphorylation [24]. Based on the mechanisms of Type II pyrethroids, some suggestions concerning the convulsant activity of cypermethrin have been made. It has been proposed that the increase in sodium conductance caused by cypermethrin, along with the decreased probability of interaction between GABA and $GABA_A$ receptor, leads to an imbalance in neural circuit activity, favouring system excitability [23]. Other observations have suggested that excitatory amino acid receptors might be a possible target for the convulsive action of cypermethrin [22]. It has been demonstrated that MK-801, an N-methyl-D-aspartate (NMDA) receptor antagonist, antagonized the convulsant action of cypermethrin administered centrally or peripherally in mice and rats [22]. It might be suggested that similar mechanisms as in the case of cypermethrin may be responsible for the convulsive effects of zeta-cypermethrin.

In a previous report, caffeine did not affect seizure activity induced by the tested pyrethroids, deltamethrin, β-cyfluthrin and tefluthrin, at the dose (40 mg/kg, i.p.) capable of increasing

the neurotoxic effects of pyrethroids (impairment of motor coordination) [9]. In the current study, caffeine at the same dose did not alter the convulsive effects of zeta-cypermethrin. It is noteworthy that caffeine at a similar dose range (23.1 – 46.2 mg/kg, i.p.) diminished the protective action of some antiepileptic drugs against electroconvulsions in mice [25, 26]. It can be concluded that antiepileptic drugs are more susceptible to interactions with caffeine than pyrethroids as regards seizure occurrence.

Zeta-cypermethrin used at its 1/5 CD_{50} (3.2 mg/kg, i.p.) did not disturb motor coordination in the rota-rod test and learning in the passive avoidance task in mice. Tefluthrin (Type I pyrethroid) applied at its 1/5 CD_{50} induced motor impairment in the rota-rod, which suggest that zeta-cypermethrin at a comparable dose range has a lower toxic potential on mouse performance in this test. Furthermore, simultaneous exposure to tefluthrin and caffeine led to the pronounced impairment of motor coordination in the rota-rod test [9], while co-exposure to zeta-cypermethrin (Type II pyrethroid) and caffeine did not influence their activities in this test, supporting the suggestion that the interaction between caffeine and pyrethroids could depend on the type of pyrethroid [9]. Other pyrethroids belonging to Type II group, deltamethrin and β-cyfluthrin, did not interact with caffeine in the rota-rod, either [9]. Therefore, a pyrethroid group-related effect for the combination with caffeine in the rota-rod cannot be excluded and the current observation support this hypothesis. Hence, some differences in the mechanisms of toxicity between Type I and Type II pyrethroids have been reported [27]. It is thought that at all organizational levels, Type I pyrethroids have qualitatively and quantitatively distinct impacts from Type II pyrethroids. These levels include the ion channel level, the macroscopic levels of organization within electrically conductive tissue (nerve-muscle, spinal cord, hippocampus, and cortical neurons), as well as the level of the entire animal, where toxicologically significant responses and lack of such effects may be detected [28].

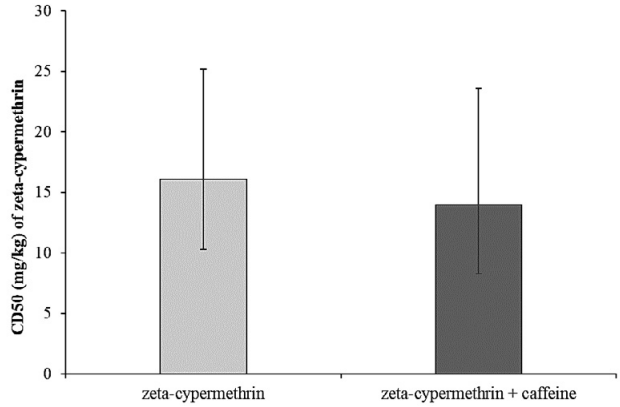

Figure 1. Effect of caffeine on seizure activity of zeta-cypermethrin in mice. Data are shown as median convulsive dose (CD_{50} in mg/kg) with 95% confidence limits. Caffeine was administered at the dose of 40 mg/kg. $p > 0.05$ vs. zeta-cypermethrin group (according to Litchfield and Wilcoxon [20])

Table 1. Effect of caffeine and zeta-cypermethrin on learning in the passive avoidance test and motor coordination in the rota-rod test in mice

Substance (mg/kg)	Retension time (s)	Motor coordination impairment (s)
control	180 (180, 180)	120 (120, 120)
caffeine (40)	180 (180, 180)	120 (120, 120)
zeta-cypermethrin (3.2)	180 (180, 180)	120 (120, 120)
zeta-cypermethrin (3.2) + caffeine (40)	180 (180, 180)	120 (120, 120)

Results are shown as median values (in seconds – s) along with the 25th and 75th percentiles. The number of mice in each group was eight. $p > 0.05$ vs. control group (Kruskal-Wallis/ Dunn's test).

CONCLUSIONS

This study shows that zeta-cypermethrin (Fury 100 EW) may exert convulsant activity in mice. The underlying mechanisms of this zeta-cypermethrin-induced neurotoxic effect need further studies. Caffeine did not interact with zeta-cypermethrin in the behavioural tests. Based on the current results, it can be suggested that co-exposure to zeta-cypermethrin and caffeine does not affect their neurotoxic potential.

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