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Research report

Two cholinesterase inhibitors trigger dissimilar effects on behavior and body weight in C57BL/6 mice: The case of chlorpyrifos and rivastigmine

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HIGHLIGHTS

- Different doses of rivastigmine induced time-dependent weight increase.
- CPF and rivastigmine inhibited brain AChE following an isoform-specific pattern.
- CPF boosted the choice of the random learning strategy at the end of the exposure.
- CPF affected spatial memory at the end of the exposure period.
- The low dose of rivastigmine improved memory recall after a washout period.

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ABSTRACT

Cholinesterases (ChE) are common targets of organophosphate (OP) pesticides and play a critical role in the pathology of some dementias. While chlorpyrifos (CPF) remains one of the most commonly used OPs in the world, numerous investigations have reported its neurotoxic potential and highlighted behavioral disturbances upon its administration. Rivastigmine currently serves to treat Alzheimer's disease, but it may induce cholinergic overstimulation in non-demented individuals. The present investigation aimed to compare the acute and delayed effects caused by both ChE inhibitors in adult C57BL/6 male mice. The animals were daily fed either a standard, a CPF- (5 mg/kg body weight) or a rivastigmine-supplemented diet (1 or 2 mg/kg body weight) for 8 weeks. After the treatment, we established an 8-week washout period to assess recovery. ChE enzyme activity, biomarkers, physical effects, and behavioral alterations were evaluated at different time points during the exposure and after the washout period. Both rivastigmine doses induced a time-dependent weight increase. CPF and rivastigmine inhibited brain acetylcholinesterase following an isoform-specific pattern. As for behavioral assessment, CPF negatively modulated learning strategies and impaired memory in a Barnes maze task at the end of the exposure. On the other hand, the low dose of rivastigmine improved memory recall at the end of the washout period in a Morris water maze. Indeed, our results endorse the positive effects of low doses of rivastigmine following a drug-free period in young mice. Therefore, doses and periodicity of treatment to improve cognition in elderly people upon rivastigmine administration should be revised.

1. Introduction

The central cholinergic system plays a critical role in numerous cognitive processes, including spatial learning and memory [1,2]. Likewise, such types of dementia as Alzheimer's disease (AD) and Parkinson disease have been associated with a cholinergic deficit

Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; AD, Alzheimer's disease; BM, Barnes maze; ChE, cholinesterase; CPF, chlorpyrifos; FDA, Food and Drug Administration; MWM, Morris water maze; OF, open field; OP, organophosphate; RIV, rivastigmine.

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[3]. One mechanism to increase the amount of synaptic acetylcholine (ACh) is the inhibition of acetylcholinesterase (AChE) [4], which justifies using cholinesterase (ChE) inhibitors to alleviate the symptoms of dementia. Specifically, rivastigmine is a carbamate that inhibits both AChE and plasma ChE for several hours [5] by pseudo-irreversibly binding to these enzymes [6]. Nowadays, it is used to treat both AD and Parkinson disease in their mild and moderate phases [5]. Traditionally, the drug has been prescribed in various formulations, including capsules, oral solution, and transdermal patch, being the latter the most clinically effective [7]. Among the beneficial effects exerted by this drug, a large volume of animal-based studies have reported that rivastigmine enhances memory [8–10].

On the other hand, organophosphate (OPs) compounds are a large class of chemicals that are extensively used worldwide as pesticides [11]. In recent decades, there has been increasing interest in relating the development of neurological disorders and cognitive deficits to exposure to pesticides. Nowadays, chlorpyrifos (CPF) is one of the most frequently used OPs to control crop damage by insects in agriculture [12]. Since CPF irreversibly binds to and inhibits both AChE and plasma ChE, cholinergic hyperstimulation occurs [13]. A huge number of studies involving experimental animals have indicated that when acutely administered CPF produces long-term behavioral alterations and neurological deficits, such as altered inhibitory control [14], spatial memory impairments [15], and changes in locomotor activity [16].

Currently, there is a growing body of literature that recognizes the importance of low-level pesticide exposures and their impact on the health of the general population. With this in view, low doses of CPF have been related to neurotoxic effects [17–19], deficits in spatial learning and inability to use newly acquired information [20–23], as well as metabolic alterations in animal models [24–26]. Likewise, a number of researchers have indicated that dietary intake is the most common source of exposure for the general population [27,28].

Structural cholinergic elements and their relevance to cognitive functions is a continuing research concern. In this line, different isoforms of brain AChE are found in various synaptic compartments. The cytosolic G1 isoform is isolated from the salt-soluble fraction and can be readily detected in such brain areas as the cortex, hippocampus and amygdala [29]. The G4 isoform, though, is bound to the membrane and isolated from the detergent-soluble fraction [29]. Further, it has been recurrently reported that the G4 isoform is more abundant than G1 in healthy subjects [30,31]. In fact, Meshorer et al. [32] have revealed a long-term replacement of the G4 isoform by the G1 under stress conditions. A broader perspective has been adopted by Farchi et al. [33], who suggested that both G4 and G1 isoforms modulate cognitive performance differently.

By drawing on the concept of harmful effects, Pope et al. [34] mentioned that the mechanism of toxicity of ChE inhibitors is essentially the same as the mechanism for therapeutic uses. In parallel, a much debated question is whether ChE inhibitors can be prescribed to patients with mild cognitive impairment, but without a declared dementia [35]. According to Pope and collaborators [34], cholinergic overstimulation under normal conditions leads to an imbalance in ACh neurotransmission, which may ultimately trigger the emergence of cholinergic signs of toxicity. Up to now, no controlled studies have compared potential differences between two or more ChE inhibitors sharing the same primary target, but with different usages.

The present study sought to investigate the differences between CPF and rivastigmine, by evaluating the acute and delayed effects on physical and other general parameters, locomotor activity and spatial learning and memory, following a subchronic low dietary exposure in C57BL/6 adult male mice.

2. Materials and methods

2.1. Animals and care

Three-month-old C57BL/6 male mice (Charles River, Barcelona, Spain) were used for this study. The animals were housed in plastic cages containing 3–5 individuals and kept under a 12-h light-dark cycle (lights off at 8 p.m.) in an environmentally controlled room, held at 22 °C ± 2 °C and a relative humidity of 50 ± 10%. The mice were allowed free access to food and tap water and given a normal chow diet (Panlab, Barcelona, Spain) before the experiment started. The use of animals and the experimental protocols were approved by the Animal Care and Use Committee of the Rovira i Virgili University (Tarragona, Spain) and were conducted in accordance with the Spanish Royal Decree 53/2013 on the protection of experimental animals and the European Communities Council Directive (2010/63/UE).

2.2. Chemical compounds

Rivastigmine tartrate ((*S*)-3-[1-(dimethylamino)ethyl]phenyl *N*-ethyl-*N*-methylcarbamate, purity 98%) was supplied by TCI Europe N.V. (Zwijndrecht, Belgium). CPF (*O*,*O*-diethyl *O*-(3,5,6-trichloropyridin-2-yl) phosphorothioate, purity 99.5%) was provided by Sigma-Aldrich (Seelze, Germany). Three diets were obtained by supplementing standard rodent chow with either CPF or rivastigmine. Previous to the treatment, basal food intake was recorded to determine the total amount of each chemical compound to be added to the standard diet (data not shown). Thus, the diet supplemented with CPF (37.5 mg CPF/kg chow) was intended to deliver a dose of 5 mg/kg body weight/day, which was expected to be below the range of acute signs of toxicity [36]. Likewise, two types of diet were manufactured to deliver either 1 (RIV1) or 2 (RIV2) mg/kg body weight/day rivastigmine (7.5 mg RIV1 and 15 mg RIV2/kg chow). According to the US Food and Drug Administration (FDA), the subsequent rivastigmine-equivalent dose in humans can be calculated using a conversion factor, which considers the body surface area and the body weight of both the human and the mouse [37]. Thus, the range of doses of the drug we used in this study (i.e., 1 and 2 mg/kg/day) corresponded to 0.08 and 0.16 mg/kg/day in humans, respectively, and match those traditionally administered in transdermal patches (i.e., 4.6 mg/24 h and 9.5 mg/24 h, respectively) [7].

2.3. Treatment and experimental design

The experimental procedure is depicted in Fig. 1. A total of 69 animals were weighed and divided into four experimental groups as follows: control (n = 15), CPF (n = 18), RIV1 (n = 18), and RIV2 (n = 18). On the basis of this classification, mice were daily provided with 4 g/mouse of either a standard, a CPF- (5 mg/kg body weight/day), or a rivastigmine-supplemented diet (1 or 2 mg/kg body weight/day) for 8 consecutive weeks. In order to ensure that the mice received the estimated dose, we monitored body weight and food consumption weekly to further calculate the real ingested doses, which were: 4.93 ± 0.22 mg/kg CPF, 0.97 ± 0.04 mg/kg RIV1, and 2.08 ± 0.08 mg/kg RIV2. Over the course of the whole study, the emergence of potential cholinergic signs was also monitored.

2.4. Behavioral assessment

The total number of animals used for each behavioral task depended on when these tasks were carried out (Fig. 1). Notwithstanding, a minimum of n = 10/group was guaranteed. The path and movements of the mice were recorded by a video camera (Sony CCD-IRIS), and then computerized by means of a video-