Chemico-Biological Interactions 290 (2018) 119–125

Contents lists available at ScienceDirect

Chemico-Biological Interactions

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

Electro-behavioral phenotype and cell injury following exposure to paraoxon-ethyl in mice: Effect of the genetic background

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ARTICLE INFO

Keywords:
Organophosphorus pesticide
Electro-behavioral phenotype
Status epilepticus
Cell injury
C57BL/6J and Swiss mice
Genetic background

ABSTRACT

Organophosphorus compounds (OP) are irreversible inhibitors of both central and peripheral cholinesterases (ChE). They still represent a major health issue in some countries as well as a terrorist and military threat. In order to design appropriate medical counter-measures, a better understanding of the pathophysiology of the poisoning is needed. Little to nothing is known regarding the impact of the genetic background on OP-induced seizures and seizure-related cell injury. Using two different mouse strains, Swiss and C57BL/6J, exposed to a convulsing dose of the OP pesticide paraoxon-ethyl (POX), our study focused on seizure susceptibility, especially the occurrence of SE and related mortality. We also evaluated the initial neuropathological response and SE-induced cell injury.

Following the administration of 2.4 mg/kg POX, more Swiss mice experienced SE than C57BL/6J (55.6% *versus* 17.2%) but the duration of their SE, based on EEG recordings, was shorter (64.3 ± 19.5 min *versus* 180.8 ± 36.8 min). No significant difference was observed between strains regarding mortality (33% *versus* 14%). In both strains limited cell injury was observed in the medial temporal cortex, the dentate gyrus and the CA3 field without inter-strain differences (Fluorojade C-positive cells/mm^2). Conversely, only C57BL/6J mice showed cell injury in the CA1 field. There was no obvious correlation between the number of Fluorojade C-positive cells and the duration of the EEG discharges.

Our work suggests some differences between Swiss and C57BL/6J mice and lay ground to further studies on the impact of strains in the development of central nervous system toxicity of OP.

1. Introduction

Organophosphorus (OP) nerve agents (OPNAs) still represent both a military and a credible terrorist threat as shown by the recent sarin attacks in Syria, the mediatized assassination of the half-brother of the North Korean leader with VX or the attempted murder on a former Russian agent and his daughter with what seems to be a new type of OPNA. A better understanding of the pathophysiology of OP poisoning would improve existing medical countermeasures. However, the use of OPNAs for research is highly regulated by the Chemical Weapons Convention (CWC) and OP pesticides (OPPs) may then represent interesting surrogates to OPNAs. They should also attract attention for themselves as they may be also used as weapons and do represent a serious health issue in numerous countries, with ca. 2 to 3 million intoxications and hundreds of thousands deaths every year all around the world [1,2]. Among these OPPs of concern is parathion, a highly toxic OPP, now forbidden in Europe.

OPs are irreversible inhibitors of both central and peripheral cholinesterases (ChE). Poisoning results in acetylcholine (ACh) accumulation and therefore in an uncontrolled activation of cholinergic synapses. This explains the acute clinical signs of peripheral and central origin such as salivation, miosis, bronchoconstriction, convulsions and even death due to cardiorespiratory failure [3]. Brain hypercholinergy initiates epileptic seizures [4] that may evolve into *status epilepticus* (SE) depending on different factors such as the type and amount of toxicant. Several experimental models of seizure have been described with paraoxon (POX), the main toxic metabolite of parathion (for a review see Ref. [5]). Despite the limited body of evidence, sex and genetic background appear to modulate the consequences of seizures in both the pilocarpine and kainate models. In the pilocarpine model, SE-

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https://doi.org/10.1016/j.cbi.2018.05.009
Received 27 February 2018; Received in revised form 14 May 2018; Accepted 21 May 2018
Available online 22 May 2018
0009-2797/

induced cell injury has been observed in both C57BL/6J and FVB/NJ strains while BALB/cJ and BALB/cByJ strains did not exhibit cell injury. Conversely, C57BL/6J seems protected against SE-induced cell injury following kainate administration [6–8]. At difference, little to nothing is known regarding the impact of these factors in OP-induced seizures and seizure-related cell injury. One may assume that some similarities may exist with pilocarpine but data are needed.

Based on the wide use of C57BL/6J in the field of experimental epilepsy [8,9] and the use of this background for genetically-modified mice, we decided to expose this strain to POX. In order to evaluate the influence of the genetic background on the central nervous system effects of a POX exposure, we also studied its effect on Swiss mice, a strain that we used previously [10]. Our study focused on mortality, seizure susceptibility, especially the relationship between EEG and behavioral changes, hereafter called electro-behavioral phenotype, and the initial neuropathological response to SE, namely neuroinflammation and SE-induced cell injury.

2. Materials and methods

2.1. Animals

All experiments using animals performed in the present study were approved by the Institutional Animal Care and Use Committees of our research centers. They complied with the current and appropriate European and French legislations, including the ARRIVE guidelines, 1986 and associated guidelines and EU Directive 2010/63/EU for animal experiments.

6 week-old male C57BL/6J and Swiss mice were provided by Janvier Labs (Le Genest-Saint-Isle, France). Animals were hosted in a facility with controlled temperature (21–23 °C) and humidity (30–50%) and with a 12 h light cycle (7 a.m.-7 pm). Food and water were given *ad libitum*. All animals were housed for 5–7 days prior to any experiments. Animals were 8 week-old when exposed to POX.

Given the importance of age in the susceptibility to develop seizure and seizure-related brain injury [11], we decided to use mice of similar age with the drawback that their average weights were significantly different. For the behavioral, EEG and cell injury studies, there was a significant difference ($p < 0.0001$) with Swiss (38.5 ± 0.6 g) being heavier than C57BL/6J mice (24.5 ± 0.4 g). A similar difference ($p < 0.0001$) was again found in the cytokine studies with Swiss (35.9 ± 0.4 g) being heavier than C57BL/6J mice (24.5 ± 0.4 g). This had consequences on the quantity (μg) of POX administered to the animals. Mice of the same weight would have been ca. 4-5 week-old (Swiss) vs ca. 8 week-old (C57BL/6J).

2.2. Chemicals

POX (1 g vial, > 90% pure) was provided by Sigma Aldrich (Saint-Louis, Missouri, USA). It was diluted to 3.5 mg/mL in di-methyl sulfoxide (DMSO) and kept at +4 °C for long term storage. Only one vial was used for all the experiments. On the day of the experiment, this stock solution was then diluted extemporaneously with 9‰ sodium chloride to obtain the working solution (5.3–14.4% v/v DMSO in final solution). POX was injected subcutaneously (s.c.) under the nape of the neck (200 μL) like in previous experiments using POX [10].

The oxime HI-6 dichloride (HI-6), a ChE reactivator used as pretreatment against the peripheral effects of POX, was a generous gift of Defense Research and Development Canada, Suffield, Canada.

Bupivacaine hydrochloride was from Mylan (Canonsburg, Pennsylvania, USA), pentobarbital sodium (Dolethal®) from Vétoquinol (Lure, France), chloral hydrate from Sigma Aldrich (Saint-Louis, Missouri, USA), isoflurane from Zoetis (Parsippany, New Jersey, USA), Fluorojade C® (AG 325) from Merck Millipore (Molsheim, France). All other chemicals were of the best chemical grade.

2.3. Surgery

After one week of acclimatization, surgery for stereo-electroencephalography (s-EEG) or electrocorticography (ECoG) was performed under anesthesia with additional local anesthesia provided by bupivacaine hydrochloride (25 mg/kg, 150 μL, s.c.).

The s-EEG surgery consisted in the implantation of a stereotaxic bipolar handmade stainless steel deep electrode in the right hippocampus (Bregma as a reference, coordinates: lateral = reference - 1.5 mm, anteroposterior = reference - 2.0 mm, dorsoventral = reference - 2.0 mm, [12]) and 3 monopolar handmade silver cortical electrodes (left/right frontal and left parietal cortex). A similar monopolar cortical electrode (cerebellum) was used as a reference. Anesthesia was obtained by the administration of a 4% chloral hydrate solution (400 mg/kg, i.p.).

Once the course of the electrophysiological events after POX exposure was evaluated, we recorded the animals with a tripolar electrode in order to assess the time to SE and its duration. Tripolar stainless steel cortical electrodes (Plastics One, MS333, Roanoke, VA, USA) were used (left/right parietal cortex and cerebellar cortex as a reference, lateral and anteroposterior coordinates similar to s-EEG surgery). Anesthesia was obtained by inhalation of isoflurane (induction: 3%, maintenance: 1–1.5%, in air).

2.4. POX exposure

One week after surgery, the POX injection was performed between 9:00 a.m. and 11:30 a.m. to take into account the circadian variations of cholinergic parameters [13]. We used 50 mg/kg of HI-6 injected i.p. 5 min prior to POX based on our previous experience with soman [14].

The first experiments were conducted to determine the amount of POX that would induce SE without causing too many fatalities. In a previous study, Swiss mice were injected with 1.8 mg/kg of POX [10]. More reproducible results are expected when using genetically characterized strains and C57BL/6J mice were chosen for our preliminary experiments. Comparison with other data obtained using this strain would also be possible [15,16]. First, non-implanted animals were injected s.c. with raising doses of POX: 1.6 (n = 8), 1.8 (n = 8), 2.0 (n = 10) and 2.2 mg/kg (n = 5), to determine the mortality in naive animals (Table 1). Then animals that were prepared for s-EEG were injected s.c. with raising doses of POX: 2.0 (n = 8), 2.2 (n = 8), and 2.4 mg/kg (n = 8) to determine the mortality and the SE rate in this

Table 1
Number of animals, POX doses (mg/kg) and strains used in the different experiments.

		Strain	POX Dose (mg/kg)					
			0	1.6	1.8	2.0	2.2	2.4
Dose finding (n)	No surgery	C57BL/6J	–	8	8	10	5	–
	Surgery (s-EEG)	C57BL/6J	–	–	–	8	8	8
Multiple electrodes EEG recording (n)		C57BL/6J	–	–	–	–	–	16
Delay and duration of SE (n)		C57BL/6J	–	–	–	–	–	29
		Swiss	–	–	–	–	–	18
Cell injury (n)		C57BL/6J	–	–	–	–	–	27
		Swiss	–	–	–	–	–	12
Neuroinflammation (n)	Proteins	C57BL/6J	9	–	–	–	–	11
		Swiss	10	–	–	–	–	8
	mRNA	C57BL/6J	10	–	–	–	–	8