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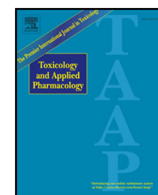

Sub-chronic exposure to the insecticide dimethoate induces a proinflammatory status and enhances the neuroinflammatory response to bacterial lypopolysaccharide in the hippocampus and striatum of male mice

Mariana Astiz *, Yolanda Diz-Chaves, Luis M. Garcia-Segura

Instituto Cajal, CSIC, Avenida Doctor Arce 37, E-28002 Madrid, Spain

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ABSTRACT

Dimethoate is an organophosphorus insecticide extensively used in horticulture. Previous studies have shown that the administration of dimethoate to male rats, at a very low dose and during a sub-chronic period, increases the oxidation of lipids and proteins, reduces the levels of antioxidants and impairs mitochondrial function in various brain regions. In this study, we have assessed in C57Bl/6 adult male mice, whether sub-chronic (5 weeks) intoxication with a low dose of dimethoate (1.4 mg/kg) affects the expression of inflammatory molecules and the reactivity of microglia in the hippocampus and striatum under basal conditions and after an immune challenge caused by the systemic administration of lipopolysaccharide. Dimethoate increased mRNA levels of tumor necrosis factor α (TNFα) and interleukin (IL) 6 in the hippocampus, and increased the proportion of Iba1 immunoreactive cells with reactive phenotype in dentate gyrus and striatum. Lipopolysaccharide caused a significant increase in the mRNA levels of IL1β, TNFα, IL6 and interferon-γ-inducible protein 10, and a significant increase in the proportion of microglia with reactive phenotype in the hippocampus and the striatum. Some of the effects of lipopolysaccharide (proportion of Iba1 immunoreactive cells with reactive phenotype and IL6 mRNA levels) were amplified in the animals treated with dimethoate, but only in the striatum. These findings indicate that a sub-chronic period of administration of a low dose of dimethoate, comparable to the levels of the pesticide present as residues in food, causes a proinflammatory status in the brain and enhances the neuroinflammatory response to the lipopolysaccharide challenge with regional specificity.

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Introduction

In the last decades, several epidemiological studies have demonstrated a link between exposure to environmental pollutants and the incidence of neurodegenerative disorders (Barlow et al., 2005; Le Couteur et al., 1999; Patel et al., 2006). Among environmental pollutants, agrochemicals are continuously used on a massive scale for global food production and persist as residues in food of both vegetal and animal origin, as well as in air and water (Bolognesi and Morasso, 2000). The evidence that many of these chemicals may be toxic in vivo at extremely low doses, suggest that permitted residue levels in food may be too high (Kapka-Skrzypczak et al., 2011; McKinlay et al., 2008).

Dimethoate (DMT) is an organophosphorus (OP) insecticide of systemic action, extensively used in horticulture for pest treatment in onions, tomatoes, and citric fruits and as an acaricide for treating gardens, vineyards, and field crops (CASAFE, 2007). Previous studies have shown that the administration of DMT to male Wistar rats, at a very low dose and during a sub-chronic period, increases the oxidation of lipids and proteins, reduces the levels of antioxidants and impairs mitochondrial function in various brain regions (Astiz et al., 2009a,b).

Although OP pesticides are known to inhibit acetylcholinesterase activity in the central (CNS) and the peripheral nervous system (Banks and Lein, 2012; Kwong, 2002), a number of observations suggest that OP neurotoxicity is not entirely due to perturbations of cholinergic systems, especially during chronic exposure (Duysen et al., 2001; Kamel and Hoppin, 2004; Rohlman et al., 2011; Zurich et al., 2004). Thus, recent studies suggest that OPs may cause disruption of a number of metabolic and cell signaling pathways that affect cell proliferation, differentiation and survival (Hargreaves, 2012; Rush et al., 2010). Furthermore, there is evidence that acute and chronic OP intoxications are associated with modifications in the basal inflammatory status and in the immune response to an inflammatory challenge (Banks and Lein, 2012; Hirani et al., 2007; Rodgers and Xiong, 1997; Sing and Jiang, 2003). The actions of OPs on the immune system are highly relevant for the brain since an altered neuroinflammatory response under pathological conditions may

* Corresponding author. Fax: +34 915854754.
E-mail addresses: marianaastiz@gmail.com (M. Astiz), ydiz@cajal.csic.es (Y. Diz-Chaves), lmgs@cajal.csic.es (L.M. Garcia-Segura).

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enhance neurodegenerative damage. Indeed, systemic infection may interact with environmental insults to induce exaggerated neuroinflammatory, neurodegenerative and behavioral changes and may have deleterious consequences for the brain when encountered in the context of concomitant chemical toxin, traumatic head injury, or psychological stressor exposure (Diz-Chaves et al., 2012, 2013; Litteljohn et al., 2011; Mangano and Hayley, 2009). We propose that environmental toxins might promote a sensitization of neural tissue that may enhance the neuroinflammatory response to a secondary immune challenge.

Thus, in this study, we have administered to male mice a low dose of DMT which is comparable to the levels of the pesticide present as residues in food. The dose was estimated on the basis of the Maximum Residue Levels (MRLs) established by European Union (http://ec.europa.eu/index_en.htm). We assessed whether sub-chronic intoxication affects inflammatory markers in the hippocampus and striatum under basal conditions and after an immune challenge caused by the systemic administration of lipopolysaccharide (LPS). Systemic administration of LPS has been used in this study since it is known that this treatment alters the levels of different inflammatory molecules in the brain (Dantzer, 2004; Kubera et al., 2011; Monje et al., 2003) and induces the activation of microglia (Tapia-Gonzalez et al., 2008).

Microglia participate in the local inflammatory response of the CNS, releasing a variety of inflammatory mediators, including cytokines such as TNF-α, IL1β and IL6, and chemokines such as interferon γ-inducible protein 10 (IP10; CXCL10) (Binukumar et al., 2011; Nakamura, 2002). In this study we have assessed the effect of DMT exposure on the expression of inflammatory molecules produced by microglia: TNF-α, IL1β, IL6 and IP10. In addition, we assessed immunoreactivity for Iba-1, a marker of microglia since changes in the morphology and/or number of these cells is indicative of an altered neuroinflammatory status.

The hippocampus and the striatum were selected for this study since intoxications with DMT are known to produce alterations in functions controlled by these structures, such as associative learning, associative memory and motor performance (Valenzuela-Harrington et al., 2012). In addition, the hippocampus and the striatum are extremely sensitive to inflammation and oxidative damage (Cerbai et al., 2012) and alterations in these brain structures are associated with important cognitive, affective and neurological disorders in humans (Karen et al., 2001; McDaniel and Moser, 2004; Ramos et al., 2006).

Methods

Chemicals

DMT was obtained as a gift from INTA (Instituto Nacional de Tecnología Agropecuaria, Castelar, Argentina) and was of analytical grade. The active ingredient was dissolved at 40% (p/v) in aqueous solution of polyethyleneglycol-400 at 25%.

Animals and treatments

Animals were handled in accordance with the guidelines presented in the UFAW Handbook on the Care and Management of Laboratory Animals and following the European Union guidelines (Council Directives 86/609/EEC and 2010/63/UE). Experimental procedures were approved by our institutional animal use and care committee. Special care was taken to minimize suffering and to reduce the number of animals used to the minimum required for statistical accuracy. C57BL/6 male mice (7 weeks old) weighing 23 ± 1.1 g were purchased to Harlan Laboratories. Upon arrival, mice were allowed to acclimatize for 10 days before starting the experiment, they were maintained under controlled conditions of temperature (25 ± 2 °C), and a normal photoperiod of 12 h dark and 12 h light, and fed with standard chow and water ad libitum. Body weight evaluation was performed every week during the experiment. Animals were randomly divided into two groups of twelve mice each, assigned as (i), vehicle group (injected i.p. with 25% polyethylene-glycol-400) and (ii), DMT-treated group (injected i.p. with 1.4 mg DMT/kg body weight dissolved in 25% polyethylene-glycol-400). All animals were injected three times a week for 5 weeks. The selection of the DMT dose was based on two known parameters. We first considered the established lethal dose 50 of DMT. The selected dose (1.4 mg/kg) is a 0.9% of LD50 which is considered low. In addition, we estimated the dose on the basis of the Maximum Residue Levels (MRLs) established by European Union (http://ec.europa.eu/index_en.htm). The selected dose of DMT for the present study is comparable with the doses used by other researchers in studies with similar treatment schedules (Ayed-Boussema et al., 2012; Farag et al., 2007).

Twenty-four hours before the end of the treatment with vehicle or DMT, each group of mice was divided in two. One of the subgroups received an i.p. injection of vehicle (phosphate buffered saline, PBS) and the other an i.p. injection of 5 mg/kg bw of lipopolysaccharide (LPS, from *Escherichia coli* 0111:B4, L2630 Sigma-Aldrich Corporation, St Louis, MO, USA) dissolved in PBS. Animals were killed 24 h after LPS or PBS administration. Therefore, four groups of animals were generated: a control group treated with vehicles for pesticide and for LPS (VEH/VEH, $n = 6$), a group treated with DMT and the vehicle for LPS (DMT/VEH, $n = 6$), a group treated with the vehicle for DMT and LPS (VEH/LPS, $n = 6$) and a group treated with DMT and LPS (DMT/LPS, $n = 6$). The dose of LPS was based on previous studies (Diz-Chaves et al., 2012, 2013; Qin et al., 2007).

Sample collection

Animals were killed by decapitation and the brains were quickly removed. The right hemispheres were immersed in 4% paraformaldehyde (Sigma-Aldrich Corporation) in 0.1 M phosphate buffer, pH 7.4 during 48 h and then rinsed with phosphate buffer and stored at −20 °C in a cryoprotective solution. From the left hemispheres the striatum and the hippocampus were dissected and stored at −80 °C.

Quantitative real-time polymerase chain reaction

Interleukin 1β (IL1β), tumor necrosis factor-α (TNF-α), interleukin (IL) 6 and interferon-γ-inducible protein 10 (IP10) mRNA expression levels were assessed in the hippocampus and striatum by quantitative real-time polymerase chain reaction. Tissue was homogenized and RNA was extracted using TRI reagent® solution (Ambion, USA). First strand cDNA was prepared from 2 μg RNA using M-MLV reverse transcriptase (Promega Corporation, Madison, WI, USA) according to the manufacturer's protocol. After reverse transcription, cDNA was diluted 1:4 and 1:8 for the target genes and 1:2000 for the endogenous control (18S). Five μL of these cDNA solutions were amplified by real-time PCR in a 15 μL volume reaction using SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA) using the ABI Prism 7500 Sequence Detection System (Applied Biosystems) with conventional Applied Biosystems cycling parameters (40 cycles of changing temperatures, first at 95 °C for 15 s and then 60 °C for a minute).

All the primer sequences were designed using Primer Express software (Applied Biosystems). IL1β: forward 5′-CGACAAAATACC TGTGGCCT-3′ reverse 5′-TTCTTTGGGTATTGCTTGGG-3′; TNF-α: forward 5′-GAAAAGCAAGCAGGCAACCA-3′ reverse 5′-CGGATCATGCTT TCTGTGCTC-3′; IL6: forward 5′-GAAACCGCTATGAAGTTCCTCTCTG-3′ reverse 5′-TGTTGGGAGTGGTATCCTCTGTGA-3′ and IP10: forward 5′-CAGGAGAATGAGGGCCATAGG-3′ reverse 5′-CGGATTCAGACATCT CTGCTCAT-3′. IL1β, TNF-α, IL6 and IP10 gene expressions were normalized to 18S as endogenous control.

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