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Prenatal Paraquat exposure induces neurobehavioral and cognitive changes in mice offspring

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ABSTRACT

In the present work, we investigated developmental toxicity of Paraquat (PQ), from the 1 st or 6th day of mating and throughout the gestation period. We have examined several parameters, including toxicity indices, reproductive performance, sensorimotor development, as well as anxiety and cognitive performance of the offspring.

Our results showed that exposure to 20 mg/kg of Paraquat during the first days of pregnancy completely prevents pregnancy in treated mice, but from the 6th day of pregnancy, an alteration in fertility and reproductive parameters was observed. In offspring, the PQ was responsible for an overall delay of innate reflexes and a deficit in motor development. All exposed animals showed a decrease in the level of locomotor activity, increased levels of anxiety-like behavior and pronounced cognitive impairment in adulthood.

These results demonstrated that Paraquat led to the onset of many behavioral changes that stem from the impairment of neuronal developmental processes in prenatally exposed mice.

1. Introduction

The mammalian central nervous system (NSC) goes through several distinct developmental stages before reaching full maturation. One of these critical stages in which the brain development is more sensitive to toxic insults is known as the "brain growth spurt (BGS)", starting from the gestational period and lasting at the first 3–4 weeks after birth in rodents (Dobbing and Sands, 1979). The BGS is characterized by a rapid increase in brain size and onset of synaptogenesis, neuronal proliferation and myelination (Davison and Dobbing, 1968). When developmental processes in the brain are disturbed or inhibited during the BGS, the potential for later repair will be compromised (Viberg et al., 2003). The developmental effects of pesticide exposures have been studied for several decades and remain a topic of considerable interest, since different studies suggest that exposure to these substances at the embryonic stage or at a very young age may influence the development of the CNS, causing cognitive and psychomotor alterations and delayed development of some circuits such as motor pathways (Ribas-Fito et al., 2003).

Paraquat (1,1′-dimethyl-4,40-bipyridinium dichloride) is a potent and widely used as herbicide. It interferes with photosynthesis and damages plant membrane proteins by the production of oxygen free radicals (Lock et al., 2001). PQ has been intensely studied, periodically assessed, and extensively evaluated for its possible risks (Yamada, 2003). In humans, accidental ingestion of PQ could result in multi-organ damage. Its toxicity has been extensively described, especially concerning its effects on the main target organs, lung, kidney, liver and heart (Satomi et al., 2004). However, recent investigations have been focused on the effect of the PQ in the brain and have reported several examples of damage in individuals exposed to lethal doses (Baltazar et al., 2014; Wu et al., 2013). Indeed, PQ can cross the blood–brain barrier, and it accumulates in different brain regions (frontal cortex, striatum, hippocampus, and cerebellum), leading to various functional disturbances such as motor activity, coordination, spatial memory, exploration levels, and anxiety (Barlow et al., 2003; Prasad et al., 2007). A number of these disturbances are in part related to a decrease in the number of dopaminergic neurons and contents of the striatal dopamine and its metabolites (McCormack and Di Monte, 2003).

The vulnerability of the developing CNS to injury is often more than that of the adult one. Indeed, exposure to neurotoxicants at gestation or during childhood has been reported to induce a variety of neurodevelopmental and neurological disorders (Costa and Giordano, 2007; Miodovnik, 2011). In addition, many experiments

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have shown that developmental neurotoxicants are not necessarily neurotoxic in adults, and thus, may not necessarily be identified as agents of concern if tests were limited to adults. However, there are important differences, including the role of maternal factors, critical periods of exposure, the transient nature of the developing nervous system and statistical considerations specific to developmental exposures (Rodier, 1994).

The majority of PQ neurotoxic effects was conducted either during adulthood or at a juvenile age, but none of these studies assessed the outcomes of prenatal exposure to this herbicide on the developing brain and on behavior. In order to gain information on the longer lasting effects of PQ transfer from dams to offspring, this study was designed to evaluate its developmental neurotoxicity. This was performed by determining the effects of prenatal PQ exposure on the neurobehavioral and cognitive changes of offspring. A range of behavioral tests was used to assess the neurotoxic effects of this prenatal exposure, according to the Guideline for Developmental Neurotoxicity Study (OECD, 1995).

2. Materials and methods

2.1. Animals

Male and female Swiss mice (8–10 weeks old) were obtained from the animal husbandry of the Faculty of Sciences, Cadi Ayyad University, Marrakech, Morocco. The animals were housed in Plexiglas cages (30 cm × 15 cm × 12 cm), under standard conditions of temperature (22 ± 2°C) and photoperiod 12 h/12 h. Food and water were available *ad libitum* throughout the period of the study. All procedures were conducted in accordance with approved institutional protocols, and with the provisions for animal care and use prescribed in the scientific procedures on living animals European Council Directive: EU2010/63. All efforts were made to minimize any animal suffering. The study was approved by the Council Committee of Research Laboratories of the Faculty of Sciences, Cadi Ayyad University, Marrakech.

2.2. Pesticide

Paraquat herbicide was used in the liquid commercial form, Gramoxone SL (paraquat concentration 200 g/l), supplied by the Syngenta crop protection company (USA), with molecular formula $C_{12}H_{14}N_2$, molecular weight of 186.253 g/Mol, melting point 175 °C.

2.3. Developmental neurotoxicity

The animals were housed in Plexiglas cages (two females and one male in each cage). After fertilization (appearance of a vaginal plug was considered as the day one of pregnancy (G0)), the males were removed from the cages and the females received then daily 0.2 ml of solutions throughout the gestational period. Pregnant females were divided into four groups; the control group (n = 10) treated with saline solution (0 mg/kg of PQ), T10 group (n = 10) treated with 10 mg/kg of the PQ from G0, treated with 20 mg/kg of the PQ from G0 and T20 group (n = 20) treated with 20 mg/kg of the PQ from G6 (Fig. 1).

2.3.1. Maternal effects

Pregnant mice were observed on a daily basis from the day of administration until parturition, in order to detect any symptoms of poisoning. In addition, several parameters of fertility and reproduction were evaluated as:

- Pregnancy index: the percentage of pregnant females, which have shown a vaginal plug.
- Deliverance index: the percentage of females delivering over pregnant animals.
- Viability index: the percentage of the number of living descendants in the fourth day of lactation relative to the number of offspring born alive.
- Lactation index: the percentage of the number of living offspring on day 21 relative to the number of offspring born alive.

The body weight of the offspring was also evaluated at the age P1, P7, P14 and P21.

2.3.2. The effects of prenatal exposure to PQ on mouse behavior

The behavioral testing was performed for all animals (control = 20; treated = 16) during morning sessions starting at 9 a.m. Some parameters were assessed during the same day, but the tests were separated by an interval of 30 min (Fig. 1).

2.3.2.1. PQ effect on behavioral development.

2.3.2.1.1. Negative geotaxis test (P5, P7 and P9). Motor coordination was examined using the negative geotaxis test, an automatic, stimulus-bound orientation movement which provides information about vestibular and/or proprioceptive functions (Fox, 1965). The time taken to make a U-turn from a head-down position on a 30° inclined plywood surface was measured.

2.3.2.1.2. Surface righting reflex test (P5, P7 and P9). This test evaluates the motor function and coordination (Fox, 1965). Each pup was placed on its back on a flat surface and released. The time required to get back on all four paws was measured. The maximum time allowed to each trial was 3 min.

2.3.2.1.3. Cliff avoidance test (P5, P7 and P9). This test allows studying the reflex development of the animal, as well as muscle strength. The pup was placed on a table edge with the forepaws and nose over the edge. The time taken to make a U- turn was noted, and each pup was tested once. The maximum time allowed per trial was 180 s.

2.3.2.1.4. Rotarod test (P23, P24 and P25). Motor ability and coordination were evaluated using a Rotarod apparatus for mice (6 cm diameter). Mice were tested for their ability to keep their balance on a textured roller, to prevent slippage of the animal, raised to a proper height of 28 cm relative to the horizontal platform with automatic fall detection, and rotating with different speeds of 10, 12 or 15 RPM. The animals were tested in one trial per day on PNDs 23 through 25. The duration of each animal's stay on the treadmill was registered, with a maximum duration of 5 min. The apparatus was cleaned after each trial.

2.3.2.2. PQ effect on adult behavior. All animals were tested on postnatal day 60 (Fig. 1), and their behavior was recorded and analyzed using Ethovision XT Noldus 8.5 video tracking program (Noldus Information Technology b.v., Wageningen, The Netherlands), connected to a video camera (JVC). The video camera was positioned 2.5 m above the arena, inside the vertical projection of a wall, covering the entire view of the arena. Tracking of the animal was based on contrast relative to the background. Two tracking points were specified: on the head and the center of gravity of the animal.

2.3.2.2.1. Open field test. This test is widely used to assess locomotor activity and emotional reactivity in rodents placed into an unknown arena (Wilson et al., 1976). Activity monitoring was conducted in a square shaped, white arena, measuring 50 × 50 × 50 cm. Mice were placed individually into the arena and monitored for 10 min by a Video camera (JVC). For each sample, the system recorded position, object, area and the status of defined events.