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Neurobehavioral effects of concurrent exposure to cesium-137 and paraquat during neonatal development in mice

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ABSTRACT

As a result of nuclear power plants accidents such as Chernobyl or Fukushima, some people were exposed to external and internal ionizing radiation (IR). Human brain is highly sensitive to IR during fetal and postnatal period when the molecular processes are not completely finished. Various studies have shown that exposure to low doses of IR causes a higher incidence of cognitive impairment. On the other hand, in industrialized countries, people are daily exposed to a number of toxicant pollutants. Exposure to environmental chemicals, such as paraquat (PQ), may potentiate the toxic effects induced by radiation on brain development. In this study, we evaluated the cognitive effects of concomitant exposure to low doses of internal radiation (^{137}Cs) and PQ during neonatal brain development. At the postnatal day 10 (PND10), two groups of mice (C57BL/6J) were exposed to ^{137}Cs (4000 and 8000 Bq/kg) and/or PQ (7 mg/kg). To investigate the spontaneous behavior, learning, memory capacities and anxiety, behavioral tests were conducted in the offspring at two months of age. The results showed that cognitive functions were not significantly affected when ^{137}Cs or PQ were administered alone. However, alterations in the working memory and anxiety were detected in mice exposed to ^{137}Cs combined with PQ.

1. Introduction

With the increased use of nuclear technologies in power production, as well as in medical and industrial applications, there is an enhanced likelihood of ionizing radiation (IR) exposure (Haridas et al., 2012; Kumar et al., 2013; Trivedi et al., 2012). The recent Fukushima Daiichi Nuclear Power Plant (FNPP) disaster has increased the interest of the scientific community about the health effects of some radionuclide species such as cesium-137 (^{137}Cs). This radionuclide was released from FNPP increasing their accumulation into terrestrial, aquatic and marine environments worldwide (Buesseler et al., 2011). Because ^{137}Cs has a longer half-life (30.07 years) compared with other radiocesium isotopes, it tends to accumulate in bottom sediments, aquatic plants and fish, being the contamination of drinking water and the consumption of contaminated plants or fish the main pathways for potential human exposure (Ashraf et al., 2013). People can be exposed to IR externally to a close source of radiation, or internally by radioactive material that has entered the body (Fushiki, 2013).

Actively dividing cells in the neurogenic areas of the brain are considered to be extremely sensitive to IR (Haridas et al., 2012). It was suggested that IR can induce impairment in the dentate subgranular zone (SGZ) of the hippocampus. Consequently, radiation-induced cognitive changes are often manifested as deficits in hippocampal-dependent functions of learning and memory (Rola et al., 2004). Effects on brain growth and development have emerged from studies at Hiroshima and Nagasaki (ICRP, 2003). Results of epidemiological studies clearly pointed toward an increased risk of mental retardation in children of the surviving women of the Hiroshima/Nagasaki atomic bombing, when *in utero* exposure occurred between weeks 8 and 15 of pregnancy or, extended between weeks 15 and 25 (Verheyde and Benotmane, 2007). Moreover, there are reports indicating radiation-induced cognitive dysfunctions in the patients undergoing radiotherapy (Dietrich et al., 2008; Dockstader et al., 2014). Pediatric patients exposed to therapeutic doses showed a persistent long term cognitive

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impairment along with the learning disabilities (Kumar et al., 2013). Although existing data are mainly based on high doses of IR (≥1 Gy), less is known about the effects of low doses of radiation. In a Swedish study (Hall et al., 2004), children irradiated for cutaneous hemangioma, when aged under 18 months, they showed a decrease in high school attendance at doses greater than 100 mGy. Moreover, Ishida et al. (2011) studied the effects of low doses of neutron exposure (^{137}Cs gamma-ray) *in utero*, reporting significant learning disabilities and changes in the locomotor activity of mice. Recently, Buratovic et al. (2014) demonstrated in mice that an IR (350 and 500 mGy), given on postnatal day 10 (PND10), which is defined as a critical period of brain development, is sufficient to cause persistently reduced cognitive function.

On the other hand, in industrialized countries, people are exposed daily to a number of toxicant pollutants. Nowadays, there is enough evidence that some of these toxicants have harmful health effects (Collotta et al., 2013). Paraquat is a highly toxic quaternary nitrogen herbicide. It is a fast acting, non-selective compound, which destroys tissues of green plants on contact and by translocation within the plant (Tsai, 2013). In humans, intentional or accidental ingestion of PQ results frequently fatal due to multiorgan failure (Dong et al., 2013; Tsai, 2013). The toxicity of PQ has been extensively described concerning its effects to the main target organs, lungs and also kidneys, liver and heart (Dinis-Oliveira et al., 2008). However, recent investigations have been focused on the effect of PQ in the brain, after several reports of brain damage in individuals exposed to lethal doses of PQ (Baltazar et al., 2014; Wu et al., 2013). Moreover, epidemiological studies have observed a relation between occupational exposure to this herbicide and a heightened risk of developing Parkinson's disease (PD) (Costello et al., 2009; Qi et al., 2014). The mechanisms proposed of PQ-induced neurotoxicity involve: induction of oxidative stress, mitochondrial dysfunction, apoptosis and autophagy, inhibition of the ubiquitin-proteasome system, induction of synucleinopathy and tauopathy (Baltazar et al., 2014). Kumar et al. (2012) reported neurotoxic effects of parenteral administration of PQ (10 mg/kg body weight) in mice. The results showed behavioral deficits and changes in the number of dopaminergic neurons and contents of the striatal dopamine and its metabolites. Recently, Hosamani et al. (2014) demonstrated the neurotoxicity of PQ in prepubertal mice. It was found that parenteral exposure to PQ, at doses of 15 mg/kg body weight, induced oxidative stress and mitochondrial dysfunctions in different brain regions.

Cognitive function has been also studied in animals exposed to PQ. Impaired spatial memory, reduced exploration levels, and increased anxiety-like levels after PQ exposure have been observed (Chen et al., 2010; Litteljohn et al., 2009). The effect and mechanism of PQ toxicity on the hippocampus of mice was studied by Chen et al. (2010). After treatment with PQ, an oxidative damage in the hippocampus was found. Also, Morris water maze tests showed that the response latency increased significantly in animals exposed to PQ.

Concurrent exposure to low doses of IR and toxics at very young age may also influence the development of central nervous system (CNS), having a potential negative impact on cognitive development during childhood (Buratovic et al., 2014; Hall et al., 2004). Taking the above into account, the aim of this study was to investigate the effects of neonatal combined exposure to ^{137}Cs and PQ on neurobehavior of mice.

2. Material and methods

2.1. Animals

All experiments were performed in pregnant C57BL/6J mice (provided by Charles River, CRIFFA, Barcelona, Spain). Mice were kept in standard animal cages under a 12 h light/dark cycle (light: 8:00–20:00 h), at a temperature of 22 ± °C and a relative humidity of 50 ± 10%, with *ad libitum* access to tap water and food (Panlab, Barcelona). The use of animals and the experimental protocol were approved by the Animal Care and Use Committee of the Universitat Rovira i Virgili (Tarragona, Catalonia, Spain) following the "Principles of Laboratory Animal Care", and were carried out in accordance with the European Union Directive 2010/63/EU for animal experiments.

2.2. Groups and treatment

Sixty female mice were randomly assigned to different experimental groups and received a single subcutaneous dose of 0.9% saline, cesium (^{137}Cs, provided by CIEMAT, Spain), and/or paraquat (methyl viologen dichloride hydrate, 856177-1G, provided by Aldrich, Spain) at postnatal day 10 (PND10). There is enough evidence that developing brain is susceptible to permanent impairment during this time window of vulnerability (Stein et al., 2002). Six experimental groups were established: control group (receiving 0.9% saline), PQ group (receiving 7 mg/kg of PQ dissolved in 0.9% saline), ^{137}Cs 4000 group (receiving ^{137}Cs with activity of 4000 Bq/kg), ^{137}Cs 8000 group (receiving ^{137}Cs with activity of 8000 Bq/kg), PQ/^{137}Cs 4000 group (receiving 7 mg/kg of PQ dissolved in 0.9% saline and ^{137}Cs with activity of 4000 Bq/Kg) and PQ/^{137}Cs 8000 group (receiving 7 mg/kg of PQ dissolved in 0.9% saline and ^{137}Cs with activity of 8000 Bq/Kg). Cesium and PQ doses were based on the results of previous investigations (Lestaevel et al., 2008; Li et al., 2012). At the age of two months, animals were submitted to the following behavioral tests: elevated plus maze (anxiety), open-field test (anxiety/activity), water maze test (learning and spatial memory) and radial maze test (learning and spatial-working memory). Animals were tested during the same light phase of the light/dark cycle.

2.3. Behavioral tests

2.3.1. Elevated plus maze test (EPM)

The plus maze test is one of the most used tests to assess anxiety-like levels of mice. It was developed to screen anxioselective effects of drugs (Lister, 1987; Pellow et al., 1985). The apparatus used for the EPM comprises two open arms (25 × 5 × 0.5 cm) across from each other and perpendicular to two closed arms (25 × 5 × 16 cm), with a center platform (5 × 5 × 0.5 cm). The small wall (0.5 cm) in the open arms is used to reduce the number of falls. The entire apparatus is 50 cm above the floor. In our experiment, mice were transported to the behavioral testing room 30 min prior the behavioral testing. Each animal was placed in the central square at the start of a 5 min session, being allowed to explore freely the environment. After every observation period and before placing the next animal, the apparatus was cleaned with 70% ethanol in order to remove olfactory cues left by the previous animal. This allows to facilitate the effectiveness of the visual cues. Performance was recorded by a video camera placed above the maze, being the data analyzed by the video tracking program Ethovision XT© (Noldus Information Technologies, Wageningen, The Netherlands). The following parameters were registered: latency to first entry into the closed arms, time spent in open arms, number of entries into the open arms and total distance traveled over the maze (Walf and Frye, 2007). Moreover, an experimenter registered the number of head dips (downward movements of the head toward the floor) (Rodgers et al., 1997).

2.3.2. Open field test (OF)

To assess the anxiety-like levels and the activity levels of the animals we used the OF test. The OF consisted in a 47 cm × 47 cm