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Exposure to low doses of ^{137}cesium and nicotine during postnatal development modifies anxiety levels, learning, and spatial memory performance in mice

Montserrat Bellés [a, b], Luis Heredia [b, c], Noemí Serra [b], José L. Domingo [b], Victoria Linares [a, b, *]

[a] Physiology Unit, School of Medicine, IISPV, Universitat Rovira i Virgili, Reus, Spain
[b] Laboratory of Toxicology and Environmental Health, School of Medicine, IISPV, Universitat Rovira i Virgili, Reus, Spain
[c] CRAMC, Research Center for Behavior Assessment, Department of Psychology, Universitat Rovira i Virgili, Tarragona, Spain

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ABSTRACT

Radiation therapy is a major cause of long-term complications observed in survivors of pediatric brain tumors. However, the effects of low-doses of ionizing radiation (IR) to the brain are less studied. On the other hand, tobacco is one of the most heavily abused drugs in the world. Tobacco is not only a health concern for adults. It has also shown to exert deleterious effects on fetuses, newborns, children and adolescents. Exposure to nicotine (Nic) from smoking may potentiate the toxic effects induced by IR on brain development. In this study, we evaluated in mice the cognitive effects of concomitant exposure to low doses of internal radiation (^{137}Cs) and Nic during neonatal brain development. On postnatal day 10 (PND10), two groups of C57BL/6J mice were subcutaneously exposed to 137-Cesium (^{137}Cs) (4000 and 8000 Bq/kg) and/or Nic (100 μg/ml). At the age of two months, neurobehavior of mice was assessed. Results showed that exposure to IR-alone or in combination with Nic-increased the anxiety-like of the animals without changing the activity levels. Moreover, exposure to IR impaired learning and spatial memory. However, Nic administration was able to reverse this effect, but only at the low dose of ^{137}Cs

1. Introduction

Humans are exposed to ionizing radiation (IR) from environmental, medical, and man-made sources (Racine et al., 2009; Spitz and Hauer-Jensen, 2014). The distribution of artificial radionuclide species in the environment depends on the sources, the release conditions, as well as the subsequent transformation processes (Ashraf et al., 2013). Among these radionuclides, 137-Cesium (^{137}Cs) is considered an important indicator of radioactive pollution in aquatic environments. Because of its chemical similarity with potassium and its solubility in water, ^{137}Cs is readily transported through the environment and food chains (Racine et al., 2009; Yasunari et al., 2011). Thus, it tends to accumulate in bottom sediments, aquatic plants and fish, being the contamination of drinking water and the consumption of contaminated plants and fish, the main pathways for potential human exposure (Ashraf et al., 2013; Franic et al., 2008). A number of studies have shown that IR can alter the normal function of some neurogenic areas (e.g. dentate subgranular of hippocampus) (Haridas et al., 2012; Heredia et al., 2016). The epidemiological studies conducted after the Hiroshima/Nagasaki atomic bombing showed an increased risk of mental retardation in those children who were exposed *in utero* to radiation (Verheyde and Benotmane, 2007). Epidemiological evidence suggests that low doses of IR produce persistent alterations in cognition if the exposure occurs at young ages. Various investigations have shown that radiation injury can cause cognitive decline, depressive behavior, and affective state disturbances following exposure to high and low doses of radiation (Casciati et al., 2016; Manda and Reiter, 2010). Therapeutic doses of radiation used for the treatment of primary and metastatic brain tumor often causes neurological side-effects, such as intellectual impairment and memory loss (Zhang et al., 2014). For children exposed to therapeutic doses of radiation, reduced learning abilities and attendance have been reported (Hall et al., 2004; Kumar et al.,

* Corresponding author. Physiology Unit, School of Medicine, IISPV, Universitat Rovira i Virgili, Reus, Spain.
E-mail address: mvictoria.linares@urv.cat (V. Linares).

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On the other hand, tobacco consumption is considered one of the most important public health challenges. Environmental tobacco smoke comprises at least 250 toxic chemical substances, including carbon monoxide, nicotine (Nic), butane, ammonia, toluene, etc (Aurrekoetxea et al., 2016; Heck, 2010; Lawler et al., 2013; Manhaes et al., 2008; Pagani, 2014). Among these substances, nicotine is the most psychoactive substance of tobacco (Liu et al., 2015). A number of studies have been conducted in order to elucidate the behavioral effects produced by nicotine (Eppolito et al., 2010; Smith et al., 2015). Studies with experimental animals have shown that acute Nic enhances learning and memory by activating nicotinic acetylcholine receptors (nAChRs) (Kim and Levin, 1996; Kutlu and Gould, 2015b). The hippocampus is one of the most important structures involved in the learning and memory processes (Gould and Leach, 2014). There is evidence that ventral hippocampus, and acetylcholine levels, are involved in some forms of memory retrieval, such as spatial memory or spatial working-memory (Gould and Leach, 2014; Liang et al., 2015; Loureiro et al., 2012). Although mechanisms of these effects remain currently still unclear, a recent investigation has demonstrated that Nic might alter hippocampal plasticity and nAChRs activation. These modifications may occur through hippocampal cell signalling modulation (hippocampal kinases and transcription factors) (Kutlu and Gould, 2016). In addition, anxiety-like levels are also affected by Nic administration. In fact, Nic acts as an anxiolytic or anxiogenic agent depending on the conditions (Picciotto, 2003; Picciotto and Kenny, 2013). On the other hand, nAChRs are expressed in the brain on both excitatory and inhibitory neurons modulating behavioral function bidirectionally. The pre-and post-synaptic locations on neurons increase the functional diverstity of nAChR effects. nAChRs located on glutamatergic neurons modulate excitatory circuitries. However, receptors located on GABAergic neurons mediate inhibitory processes (Kutlu and Gould, 2015a).

Neurodevelopmental exposure to toxicants impairs neonatal orientation, attention, motor activity, and executive function (Schneider et al., 2011). There are clear evidences that brain irradiation can induce cognitive dysfunctions and cerebrovascular disorders. The biological processes responsible for these abnormalities are still unclear. On the other hand, confounding factors, such as environmental toxins, may increase genetic predisposition to the development of cerebral and cerebrovascular damage after radiation. Therefore, concurrent exposure to radiation and toxicants at very young ages may also influence the development of central nervous system (CNS), and could have a negative impact on cognitive development during childhood (Buratovic et al., 2014; Hall et al., 2004). Taking the above into account, this study was aimed at investigating the synergistic effects of combined neonatal exposure to ^{137}Cs and Nic on neurobehavior of the offspring.

2. Material and methods

2.1. Animals

All experiments were performed in pregnant C57BL/6J mice (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 municipal tap water and food (Panlab rodent chow, Panlab, Barcelona). The Animal Care and Use Committee of the Universitat Rovira i Virgili (Tarragona, Catalonia, Spain) approved the use of animals and the experimental protocol by following the "Principles of laboratory animal care", being carried out according to the European Union Directive 2010/63/EU for animal experiments.

2.2. Treatment

Sixty female mice were randomly assigned to different experimental groups (n = 10/group) and received a single subcutaneous dose of 0.9% saline solution, ^{137}Cs (CIEMAT, Madrid, Spain) and/or Nic ((−)-Nicotine hydrogen tartrate salt, N5260-25G, Sigma, Barcelona, Spain) on 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 (0.9% saline solution), Nic group (100 μg/ml of Nic dissolved in 0.9% saline), Cs 4000 group (^{137}Cs with activity of 4000 Bq/kg), Cs 8000 group (^{137}Cs with activity of 8000 Bq/kg), Nic/Cs 4000 group (100 μg/ml of Nic dissolved in 0.9% saline, and ^{137}Cs with activity of 4000 Bq/kg), and Nic/Cs 8000 group (100 μg/ml of Nic dissolved in 0.9% saline and ^{137}Cs with activity of 8000 Bq/kg). The 137Cs doses were selected based on the previous investigations who worked with a concentration of 137Cs similar to the maximum dose ingested by the population living in contaminated areas after the Chernobyl nuclear accident (Grignard et al., 2010; Grison et al., 2012; Handl et al., 2003; Lestaevel et al., 2008). The Nic doses were lower than on previous studies that worked with similar doses to those reported in habitual cigarette smokers (Gritz et al., 1981; Pauly et al., 2004). Those studies suggested to use lower concentrations of Nic to mimic low levels of Nic seen following second hand cigarette exposure (Pauly et al., 2004). Moreover, the Nic dose selected was indicated on previous experiments as not aversive to mice (Heath and Picciotto, 2009; Mesa-Gresa et al., 2013; Pauly et al., 2004) and in the range of the Nic doses usually employed in oral Nic treatments (Caldarone et al., 2008; Salas and De Biasi, 2008), possibly inducing significant behavioral changes in mice.

At the age of two months, animals were submitted to the following behavioral tests: Open field test (anxiety/activity), Elevated Plus Maze (anxiety), Water Maze Test (learning and spatial memory) and Radial Maze test (learning and spatial-working memory). For each behavioral domain, the less anxiogenic test was administered first (McIlwain et al., 2001). There were 24 h between tests. Animals were tested during the same light phase of light/dark cycle.

2.3. Behavioral tests

2.3.1. Elevated plus maze test (EPM)

One of the most used mazes to assess anxiety-like levels in mice is the plus maze test. It was developed to screen anxioselective effects of drugs. The apparatus used for the EPM has two closed arms (25 × 5 × 16 cm) across from each other and perpendicular to two open arms (25 × 5 × 0.5 cm), with a centre platform (5 × 5 × 0.5 cm). In the open arms, the small wall (0.5 cm) is used to decrease the number of falls. The entire apparatus is 50 cm above the floor. Mice were transported to the testing room 30 min prior to conduct the behavioral tests. Each animal was placed in the central square at the start of 5 min session and it was allowed to explore freely the environment. In order to remove olfactory cues left by the previous animal, the apparatus was cleaned with 70% ethanol after every observation period, and before placing the next animal. 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 travelled over the maze (Heredia et al., 2016; Walf and Frye, 2007). Moreover, an experimenter registered the number of head dips (downward