Internal Low Dose Ionising Radiation in Pregnant Mice: Behavioral Effects in the Offspring

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Abstract

Most of the population living in areas close to the Chernobyl accident have been exposed to external and internal low doses of ionising radiation. High doses effects are well known, however low dose radiations had not been yet investigated. The aim of this work was to investigate the cognitive effects of internal low doses ionising radiation. We evaluate the behaviour in mice pups after that pregnant females were exposed to an acute internal low dose of ^{137}Cs (ionising radiation). ^{137}Cs was administrated on GD12 through the drinking water at radiation doses of 0, 500, 1000, 2000, 4000 and 8000 Bq/Kg. Behavioural tests were conducted at the age of 2 months to evaluate the spontaneous behavior, learning, memory capacities and anxiety. Although we observed an influence of the low dose radiation, in general terms, we have not found significant changes between groups in the different tests.

Keywords. ^{137}Cs, internal low dose radiation, behavioral tests, mice.

Introduction

After the Chernobyl disaster, the population living in the contaminated areas was subjected to two types of radiation exposure: external radiation caused by the surface contamination of the environmental and internal contamination caused by food and water consumption. The main contributor to the exposure of people living in these areas is ^{137}Cs. Its chemical properties and especially the high solubility in water allow the diffusion of ^{137}Cs into plants and animals, resulting in the contamination of food chain in the years following the Chernobyl accident [1]. The biological consequences on the health status for internal exposure to ^{137}Cs are not completely understood. Several authors have described behavioural disorders and diseases of the central nervous system in populations living in radio contaminated areas.

It was generally admitted that ^{137}Cs must not have effect on the central nervous system, except perhaps at very high doses [2]. The brain is highly sensitive to ionising radiation during foetal and early post-natal period. That interval corresponds to the time of rapid proliferation and neuroblast migration from proliferative zones to the cerebral cortex. Furthermore, the risk of mental retardation is at least five-fold greater when foetuses are exposed during this period [3]. The evaluation of neurobehavioral function failed to identify significant effect of chronic exposure to ^{137}Cs [4]. Nevertheless, significant modifications in the metabolism of some neurotransmitters have been observed in rats fed with oats contaminated with ^{137}Cs at a very low dose (45 Bq/kg) for 1 month [5]. The aim of this study was to investigate the impact of internal low doses of ^{137}Cs during pregnancy on the postnatal development and behaviour of the offspring.

Methods

Animals and treatment

Pregnant female C57Bl6 mice (30 g) were randomly divided into 6 groups of 10 animals per group. At the GD12, animals of each group received a ^{137}Cs at oral doses of 0, 500, 1000, 2000, 4000 and 8000 Bq/Kg. After weaning, the pups were separated from the mothers and were raised up. The use of animals and the experimental

protocol were approved by the Animal Care and Use Committee of the "Rovira i Virgili" University (Tarragona, Spain).

Experimental setup

At the age of 2 month, there were randomly selected 8 mice of each group of dose and the following behavioral tests were carried out: open–field activity, morris water maze test, radial arm maze and elevated plus maze.

Behavioral tests

Open-field activity. General motor activity was measured in an open-field apparatus. Mice were allowed to move freely around the open-field and to explore the environment for 15 min, divided into 3 sessions of 5 min each. The path of the animals was recorded by a video camera (Sony CCD-IRIS model) that was placed above the square and was connected to a VHS videocassette recorder (Panasonic AG-5700 model). The video tracking program Etho-Vision® XT from Noldus Information Technologies (Wageningen, The Netherlands) was used to measure the total distance traveled and the number of rearings as a measure of vertical activity [6].

Morris water maze test. Spatial learning and retention were tested in a water maze according to a test modified from the procedure of Morris (1984). The animals were tested in blocks of five trials during three consecutive days (each trial started from one of four points assigned on different arbitrary quadrants of the circular tank). The trial latency to reach the platform was 60s and the intertrail interval was 60s. Once the mice reach the platform, it remained there for 30s. If the mice did not locate the platform within 60s, the animal was then placed on the platform for 30s.

The movements of the animal in the tank (160 cm diameter and 60 cm height) were monitored with a video tracking system (EthoVision® XT, Noldus Information Technology, Wageningen, The Netherlands), were measured and recorded the total distance traveled and the latency time before reaching the platform.

Probe test. At the fourth day, a single probe trial was conducted. The platform was removed from the pool and each mice was allowed to swim for 60 seconds in the maze. It was recorded the time and the distance that the animal swam in the quadrant that was supposed to have the platform [7].

Radial arm maze. In this maze was measured the working memory. It has a circular platform (diameter 18 cm) and eight arms (6 cm wide and 35 cm long) radiating from it. At the final of each arm, they have a small food pellet (5 mg) behind a low barrier preventing the animal from seeing. The animals were tested on 3 consecutive days, one trial per day. The mice had free access to water but were deprived food 12h before the initial trial. The first 2 days of the radial arm maze was used to accustom the mice to the test environment and to the maze itself. Only data from the final performance day were used for analyses. The start of each trial began with the mouse placed on the central platform always facing the same direction. The trial was terminated after 10 min or as soon as the mouse had eaten all eight food rewards. To perform well at this task, the mice had to store information continuously about which arm(s) had already been visited during a particular trial and which had not (working-memory, storing trial-specific information). The behavioral were monitored with tracking system (EthoVision® XT) and it were measures the time to find all eight pellets and the number of errors. Error is defined here as reentering an arm where the food pellet had already been devoured.

Elevated plus maze. Anxiety-like behavior was measured in this test. This maze had two opposite open arms and two opposite closed arms placed 50 cm above the floor. The animals are transferred to the testing laboratory in their home cages at least 30 min before the test. A mice was placed on the central platform facing either of the closed arms. During 5 min were measured the number of entries into the open and enclosed arms and the time spent there. The animal's behavior was recorded with a video camera (Sony CCD-IRIS model) and was used the video tracking program EthoVision® XT.

Statistical analysis

Homogeneity of variances was analyzed using the Levene's test. If variances were homogeneous, ANOVA was used followed by the Tukey method to evaluate all dose groups simultaneously. If the variances were not homogeneous, the Kruskal–Wallis test was used. Differences between groups were analyzed using the Mann–Whitney U-test. The level of statistical significance for all tests was established at $p < 0.05$. All data were analyzed by means of the statistical package SPSS 15 (SPSS Sciences, Chicago, USA).

Results

The results of the present study indicate that, in general terms, exposure of female mice to low doses of cesium during gestation did not cause relevant dose-related adverse effects on the behavior of the offspring.

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