NeuroToxicology 45 (2014) 48–55

Contents lists available at ScienceDirect

NeuroToxicology

Neonatal exposure to a moderate dose of ionizing radiation causes behavioural defects and altered levels of tau protein in mice

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ARTICLE INFO

Article history:
Received 5 September 2014
Accepted 18 September 2014
Available online 26 September 2014

Keywords:
Neonatal
Brain development
Ionizing radiation
Behaviour
Tau

ABSTRACT

Medical use of ionizing radiation (IR) has great benefits for treatment and diagnostic imaging, but procedures as computerized tomography (CT) may deliver a significant radiation dose to the patient. Recently, awareness has been raised about possible non-cancer consequences from low dose exposure to IR during critical phases of perinatal and/or neonatal brain development.

In the present study neonatal NMRI mice were whole body irradiated with a single dose of gamma radiation (0; 350 and 500 mGy) on postnatal day 10 (PND 10). At 2 and 4 months of age, mice of both sexes were observed for spontaneous behaviour in a novel home environment. The neuroproteins CaMKII, GAP-43, synaptophysin and total tau in male mouse cerebral cortex and hippocampus were analysed 24 h post-irradiation and in adults at 6 months of age exposed to 0 or 500 mGy on PND 10.

A significantly dose-response related deranged spontaneous behaviour in 2- and 4-month-old mice was observed, where both males and females displayed a modified habituation, indicating reduced cognitive function. The dose of 350 mGy seems to be a tentative threshold. Six-month-old male mice showed a significantly increased level of total tau in cerebral cortex after irradiation to 500 mGy compared to controls. This demonstrates that a single moderate dose of IR, given during a defined critical period of brain development, is sufficient to cause persistently reduced cognitive function. Moreover, an elevation of tau protein was observed in male mice displaying reduced cognitive function.

1. Introduction

The use of ionizing radiation (IR) in treatment and medical diagnostic procedures has increased over the past decade and is now the major artificial source of IR exposure (Bernier et al., 2012). Although computerized tomography (CT) scans only make up a fraction of all X-ray examinations annually performed, it has come to represent 40–67% of the received medical radiation dose in the population (Mettler et al., 2000; Bernier et al., 2012) A British epidemiological study estimated the absorbed dose of a single brain CT for children under the age of 10 years, in 2001–2008, to be approximately 30 mGy (Pearce et al., 2012b). In the USA, between the years of 1998 and 1999, children below the age of 15 were subjected to approximately 11% of all performed CT scans, of which around 50% were directed towards the cranial area. CT scans of the head region contributed 13.9% of the total effective dose in diagnostic radiology with an average effective dose of 1.5 mSv/procedure (Mettler et al., 2000). Although much attention has been focused on potential late cancer risk following exposure to IR in children for treatment of brain tumours, exposure at very young age may also influence the development of the central nervous system (CNS). An epidemiological study indicated that exposure to IR, during early human development can have a negative impact on cognitive development during childhood (Hall et al., 2004).

In many mammalian species the newborn period coincides with a period of rapid growth and development of the brain, the 'brain growth spurt' (BGS) (Davison and Dobbing, 1968). In humans, the 'BGS' begins during the third trimester of gestation and continues throughout the first 2 years of *ex utero* life. In mouse and rat this period is neonatal, spanning the first 3–4 weeks of life, during which the brain undergoes several fundamental developmental phases, viz. axonal and dendritic outgrowth, the establishment of neural connections, acquisition of new sensory and motor faculties (Bolles and Woods, 1964), resulting in a peak in

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http://dx.doi.org/10.1016/j.neuro.2014.09.002
0161-813X/

spontaneous behaviour, and numerous biochemical changes that transform the feto-neonatal brain into that of the mature adult (Davison and Dobbing, 1968; Campbell et al., 1969; Abreu-Villaca et al., 2011).

During this period of rapid brain development in mice, a distinct ontogeny of certain neuroproteins can be observed (Viberg et al., 2008; Viberg, 2009). CaMKII is known to be one of the most abundant protein kinases and is believed to play a crucial role in dendritic arborisation, long-term potentiation, memory and learning (Erondu and Kennedy, 1985; Lisman et al., 2002; Yamauchi, 2005). Growth-associated protein 43 (GAP-43) is most commonly found in the growth cone of axons and is also believed to be important for long term potentiation (Benowitz and Routtenberg, 1997; Oestreicher et al., 1997). Synaptophysin is believed to be involved in neuronal plasticity by regulating cycling and formation of synaptic vesicles (Sarnat and Born, 1999). Tau is a member of the microtubule-associated protein family which functions to stabilize and maintain a normal morphology of neurons, establish polarity and support the outgrowth of neural processes (Wang and Liu, 2008). Elevated levels of the phosphorylated tau isoform have been observed to impair normal memory and learning functions in humans and it is therefore used as a diagnostic marker in the clinic for diagnosing Alzheimer's disease.

In a previous study irradiation of neonatal mice to a single dose of 500 mGy was shown to induce persistent alterations in adult male mouse spontaneous behaviour as well as a reduced memory and learning capacity, when the exposure occurred during a critical period of the BGS (Eriksson et al., 2010).

The present study was conducted to investigate the effect after neonatal exposure to a single moderate dose of gamma radiation on (1) spontaneous behaviour and habituation to a novel home environment in adult male and female mice and (2) levels of important neuroproteins in cerebral cortex and hippocampus of neonatal and adult male mice.

2. Materials and methods

2.1. Animals

Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), after approval from the local ethical committees (Uppsala University and the Agricultural Research Council) and by the Swedish Committee for Ethical Experiments on Laboratory Animals. Pregnant Naval Medical Research Institute (NMRI) mice were purchased from Scanbur, Sollentuna, Sweden. The animals were housed individually in plastic cages in a room with an ambient temperature of 22 °C and a 12/12 h constant light/dark cycle. Animals were supplied with standardized pellet food (Lactamin, Stockholm, Sweden) and tap water ad libitum. Females were checked for birth twice daily (08.00 and 18.00 h) and day of birth was designated day 0. Within the first 48 h after birth, litter sizes were adjusted to 10–12 pups of both sexes by euthanizing excess pups. At approximately 4 weeks of age, male and female offspring were separated with regard to sex and raised in sibling groups of 3–7 individuals in separate male and female rooms.

2.2. Irradiation

Mice of both sexes were whole body gamma-irradiated on postnatal day 10 to a single dose from a ^{60}Co source at The Svedberg laboratory, Uppsala University, Uppsala, Sweden (Eriksson et al., 2010). Mice were placed in plastic dishes and exposed to a single dose of 350 or 500 mGy with a surface dose rate of about 0.02 Gy/minute. An ionization chamber (Markus chamber type 23343, PTW-Freiburg) was used to measure the dose, which was homogeneous ±3%, over the 10 cm in diameter large dish area. Control mice were placed in the same plastic dishes as the irradiated mice and sham irradiated. Animals were not anesthetized during irradiation. Each exposure group comprised mice from 3 to 4 different litters.

2.3. Spontaneous behaviour

Spontaneous behaviour, in a novel home environment, was observed in mice of both sexes at 2- and 4-months of age. The observations and recordings were carried out between 08.00 and 13.00 h, under the same light and temperature conditions as their housing conditions. During 60 consecutive minutes an automated system recorded the motor activity of the animals, and recordings of the variables locomotion, rearing and total activity were made (Rat-O-Matic, ADEA Electronik AB, Uppsala, Sweden) as described by Fredriksson (1994). Twelve cages, placed in individual soundproof boxes with separate ventilation were used.

Locomotion: Movements made in the horizontal plane were registered by the low level (10 mm above the bedding material) infrared beams.
Rearing: Movements made in the vertical plane were registered by the high level (80 mm above the bedding material) infrared beam.
Total activity: A needle mounted on a horizontal arm with a counterweight connected to the test cage registered all vibrations such as movements, grooming and shaking.

All data were collected electronically through a computer interface. The animals were observed for a 60 min period of time (0–60 min) divided into three 20 min intervals; in each 60-min session animals from each exposure group were represented. A total of 12 males and 12 females per exposure group, where 3–4 individuals were taken randomly from at least 3 different litters, were observed for spontaneous behaviour.

2.4. Slot Blot analysis

Male mice irradiated to a dose of 500 mGy were used in the Slot Blot analysis as they showed an altered spontaneous behaviour and lack of habituation. Sham irradiated mice were used as controls. The mice were sacrificed by cervical dislocation 24 h post-irradiation or at 6 months of age. The brains were dissected on an ice-cold glass plate. Cerebral cortex and hippocampus were collected, snap frozen in liquid nitrogen and stored at −80 °C until assayed. Both brain regions were homogenized in a RIPA cell lysis buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 20 mM sodium pyrophosphate, 2 mM sodium orthovanadate, 1% sodium deoxycholate) to which a 0.5% protease inhibitor cocktail (Protease Inhibitor Cocktail set III, Calbiochem) was added. Homogenates were centrifuged at 14,000 × *g* at 4 °C for 15 min and supernatant analyzed for protein content by using the BCA method (Pierce). Homogenates were stored at −80 °C. Viberg and co-workers have previously evaluated the specificity of antibodies CaMKII (Upstate Millpore, 05-552), GAP-43 (Chemicon Millipore, AB5220), synaptophysin (Calbiochem, 573822) and tau (Santa Cruz, 32274) by Western blot procedure with satisfactory results (Viberg et al., 2008; Viberg, 2009). The antibody used against tau recognizes both the non-phosphorylated and phosphorylated protein forms. Four μg of protein for CaMKII and GAP-43, 3 μg for synaptophysin and 3.5 μg for tau were diluted in sample buffer (120 mM KCl, 20 mM NaCl, 2 mM $NaHCO_3$, 2 mM $MgCl_2$, 5 mM HEPES, pH 7.4, 0.05% Tween-20, 0.2% NaN_3) to a final volume of 200 μl. Duplicates of each sample were applied to a nitrocellulose membrane (0.45 μm,