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Laboratory Investigation

LABORATORY INVESTIGATION

Effects on adult cognitive function after neonatal exposure to clinically relevant doses of ionizing radiation and ketamine in mice

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Abstract

Background: Radiological methods for screening, diagnostics and therapy are frequently used in healthcare. In infants and children, anaesthesia/sedation is often used in these situations to relieve the patients' perception of stress or pain. Both ionizing radiation (IR) and ketamine have been shown to induce developmental neurotoxic effects and this study aimed to identify the combined effects of these in a murine model.

Methods: Male mice were exposed to a single dose of ketamine (7.5 mg kg^{-1} body weight) s.c. on postnatal day 10. One hour after ketamine exposure, mice were whole body irradiated with 50–200 mGy gamma radiation (^{137}Cs). Behavioural observations were performed at 2, 4 and 5 months of age. At 6 months of age, cerebral cortex and hippocampus tissue were analysed for neuroprotein levels.

Results: Animals co-exposed to IR and ketamine displayed significant ($P \leq 0.01$) lack of habituation in the spontaneous behaviour test, when compared with controls and single agent exposed mice. In the Morris Water Maze test, co-exposed animals showed significant ($P \leq 0.05$) impaired learning and memory capacity in both the spatial acquisition task and the relearning test compared with controls and single agent exposed mice. Furthermore, in co-exposed mice a significantly ($P \leq 0.05$) elevated level of tau protein in cerebral cortex was observed. Single agent exposure did not cause any significant effects on the investigated endpoints.

Conclusion: Co-exposure to IR and ketamine can aggravate developmental neurotoxic effects at doses where the single agent exposure does not impact on the measured variables. These findings show that estimation of risk after paediatric low-dose IR exposure, based upon radiation dose alone, may underestimate the consequences for this vulnerable population.

Keywords: cognition; gamma rays; ketamine; mice; tau proteins

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Editor's key points

- Sedation or anaesthesia is commonly used to facilitate radiotherapy in children.
- Both ketamine and radiation exposure in the neonatal period can impact on subsequent cognitive function.
- In this study, neonatal mice were co-exposed to ketamine and low dose radiation equivalent to CT scanning.
- Altered habituation, learning and memory was seen in co-exposed but not single agent exposed mice, which persisted into adulthood.
- The combination of anaesthesia and radiotherapy may be more harmful than previously realized.

Exposure to ionizing radiation (IR) in the medical field for imaging purposes, diagnostics and screening or radiotherapy for tumour treatment has come to represent the major source of exposure in the general population.[1–3] In paediatric patients undergoing radiotherapy, sedation/anaesthesia before or during the procedure is often used to relieve anxiety or to prevent movement. A recent study showed that all patients ≤3 yr and around half those aged 7–8 yr were either sedated or under general anaesthesia during radiotherapy.[4] Ketamine is commonly used for anaesthesia during paediatric neurosurgery[5] and is also used as an effective analgesic or sedative agent.[5–7]

There is a specifically vulnerable period during the foetal/new-born brain development, characterized by synaptogenesis, dendritic arborization, extensive myelinization and biochemical changes, called the 'brain growth spurt' (BGS).[8] In mouse and rat, the BGS is neonatal and spans the animals first 3–4 weeks of life. During this time, novel sensory and motor faculties are acquired which together with biochemical changes result in a peak in spontaneous behaviour.[8,9]

The authors recently reported dose-response modification of behaviour in mice of both sexes, after a single neonatal IR dose of 0–1000 mGy, such that a tentative threshold neonatal whole body gamma irradiation dose for induction of developmental neurotoxicity was proposed to be around 350 mGy.[10–12] Furthermore, acute biochemical alterations in synaptophysin and tau levels and late tau level alterations has been observed in the cerebral cortex after neonatal irradiation to 500 mGy.[10] Neonatal exposure to ketamine has been shown to cause acute apoptotic neurodegeneration, alterations of essential neuroproteins, and induction of hyperactive phenotype accompanied with cognitive impairment in mice.[13–15]

The aim of this study was to explore whether IR doses in the range of the exposure associated with computerized tomography (CT) scans could interact with low doses of ketamine to induce neurotoxicity in the developing neonatal mouse brain. Neurotoxicity was evaluated by measuring cognition and habituation capacity in the spontaneous behaviour task, spatial learning abilities in the Morris water maze (MWM), and biochemical measures.

Methods

Animals

Experiments were carried out in accordance with the European Communities Council Directive of September 22, 2010 (2010/63/EU), after approval by local Ethical Committees (Uppsala University and Agricultural Research Council), the Swedish Committee for Ethical Experiments on Laboratory Animals (C37/13), and results are reported in line with relevant aspects of the Animal Research: Reporting of In Vivo Experiments Guidelines.[16] Pregnant female Naval Medical Research Institute (NMRI) mice were purchased from Scanbur (Sollentuna, Sweden) and were housed in Makrolon® III cages containing bedding and nesting material, in a room with an ambient humidity of 50–60% and temperature of 22°C, 12 h constant light–dark cycle, supplied with standardized pellet food (Lactamin, Stockholm, Sweden) and tap water *ad libitum*. Only male offspring were used in this study, to be able to compare outcomes with previous data from observations on general developmental neurotoxic effects and specific neurotoxicity of gamma radiation or ketamine in male mice.[10,12–15,17] Female littermates were culled by cervical dislocation.

Exposure

Groups of mice were exposed on postnatal (PND) 10 as follows: (1) injected s.c. with 0.9% saline [10 ml kg^{-1} body weight (b.w.)] and sham-irradiated; or (2) injected s.c. with 0.9% saline (10 ml kg^{-1} b.w.) and after 1 h, a single dose of 50, 100 or 200 mGy external whole body gamma radiation from a ^{137}Cs source [Gammacell 40 Exactor (MDS Nordion, Kanata, Canada), dose-rate 0.2 Gy min^{-1}]; or (3) a single s.c. injection of 7.5 mg kg^{-1} b.w. ketamine (Ketalar, 10 mg ml^{-1}; Pfizer Inc., New York, USA) and after 1 h sham-irradiated; or (4) co-exposed to 7.5 mg kg^{-1} b.w. ketamine, as a single s.c. injection, and after 1 h to 50, 100 or 200 mGy gamma radiation. No sedation/anaesthesia or restraint was used during the exposure. The IR and ketamine doses are based on previous studies where no/minor effect of the single compounds was observed on spontaneous behaviour, learning and memory, or levels of neuroprotein.[10–12,14,18,19]

Behavioural tests

Spontaneous behaviour

Spontaneous behaviour in a novel home environment measures the integration of sensory input into motor output and tests the animals' ability to integrate new information with information previously attained, and thereby the animals ability to habituate.[12,21] Habituation is defined as a decrease in registered counts for the measured variables: locomotion, rearing, and total activity.

Mice were observed for spontaneous behaviour in a novel home environment as previously described by Eriksson and colleagues.[12] The test was conducted at 2 and 4 months of age and observations took place between 08:00 and 12:00 h under the same light and temperature conditions in which the animals were housed. Three to four individuals were randomly chosen from three to four different litters in each exposure group and control group (n=12/exposure for all exposure groups, except 100 mGy + ketamine where n=10). In each 60-min observation session, recordings of the variables, locomotion, rearing, and total activity were made (Rat-O-Matic, ADEA Elektronik AB, Uppsala, Sweden).[12]

Morris water maze

Mice exposed to ketamine (7.5 mg kg^{-1} b.w.) (n=12), irradiated with 100 (n=12) or 200 mGy (n=12), and co-exposed to