Quantitation of brain metabolites by HRMAS-NMR spectroscopy in rats exposed to sublethal irradiation.

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Puropose : In the event of an acute total-body irradiation, whatever it is therapeutic or accidental, the physiopathology explaining the long-term neurological effects is unknown. We have developed a model of adult rats for which frequent behavioral assays were performed before and after a non-lethal whole-body ionizing radiation (60Co, 4,5 Gy). Learning and memory processing is an aspect of cognition involving mainly the hippocampus. We used high-resolution magic angle spinning (HRMAS) 1H NMR spectroscopy to characterize the biochemistry of four specific brain regions. The biological data for each animal will be compared to their behavioral performances, in order to underline any possible correlations. The best understanding of the physiopathological process in the Central Nervous System (CNS) will allow determining some prevention means or some enhancements for the radio-induced neurological late effects.

Experimental procedures: Twenty male Wistar rats were experimented for each sample period, among which ten were gamma radiated (4.5 Gy). The cerebral structures (cortex, striata, anterior and posterior hippocampus, hypothalamus) were removed at three times: 48 hours, 8 days and 30 days after radiation. The HRMAS 1H NMR experiments were performed on a Brüker DRX Avance spectrometer at 9.4 T. Samples were spun at 4 kHz and the temperature maintained at 4°C. A spin-echo sequence with a 30ms total echo time was used. Eighteen metabolites were included in the basis. They were quantitated using the quest procedure of JMRUI software, and statistically analyzed. Moreover, another group of animals was radiated and tested in the same conditions. Then a immuno-histological study of apoptosis and neurogenesis events in the CNS was made at the same removal times. Results: NMR HRMAS results present significant differences ($p<0.05$) between the radiated group and the non-radiated one. GPC decreased at 48 hours post-radiation whereas Cho and PC increased, potentially with relation to a cerebral oedema. At Day-8, a decrease of Gly and Tau, and an increase in Gln, are observed in the posterior hippocampus. One month after total body irradiation, we observe an increase of GABA in cortex and striatum. Perspectives: The behavioral data, showing a significant difference in the cognitive capacities of the rats between the radiated group and the witnesses at one month, may suggest that relevant correlations are possible with biochemical and morphological modifications of the CNS. For example, the increase in GABA levels in cortex and striatum might explain the least performances of learning test.

Article published by EDP Sciences and available at http://www.radioprotection.org
or http://dx.doi.org/10.1051/radiopro:2008647