STUDY OF COGNITIVE PARAMETERS IN POSTRADIATION PERIOD IN WHITE MICE

[1]Kalmakhelidze S.L., [1]Museridze D.P., [2]Sanikidze T.V., [3]Gogebashvili M.E., [4]Tophuria D.M., [3]Ivanishvili N.I. [3]Ormotsadze G.L.

[1]Laboratory of Neurotoxicology, I. Beritashvili Center of Experimental Biomedicine
[2]Department of Medical Physics and Biophysics, Tbilisi State Medical University
[3]Laboratory of Radiation Safety Problems, I. Beritashvili Center of Experimental Biomedicine
[4] Department of Normal Anatomy, Tbilisi State Medical University

ABSTRACT

The aim of this study is to identify a dose-and time-dependent correlation betweengammairradiation (5 Gy) inducedcognitive parametersand determine role of irradiation in aging process.Using a laboratory whitemouse model, we showed that ionizing radiation exposures causes spatial memory and behavior changes in different age groups of animals.Study revealed Instant reactions of post-radiation recovery and specificity of long term effects after one year of irradiation.Study of cognitive parameters reveled that gamma irradiation decreases spatial learning process and causes radiation aging, what consolidates the contemporary evidence that radiation can accelerate aging and mortality.

Key words: Gamma-irradiation, white mice, cognitive parameters

INTRODUCTION

Ionizing radiation has multiple effects on the brain, behavior and cognitive function. These changes are largely dependent on the radiation dose. Studies revealed that ionizing radiation affects the functions of the central nervous system what results in behavior and memory changes.These changes occur as a result of a direct irradiation of the central nervous system and also indirectly asa response to irradiation of other organ systems [1]. Dysfunctionof the central nervous system is manifested after the period of low doses radiation exposure. Nowadays, there is an increasing number of evident literature that the response of the central nervous system to the radiation is a continuous and interactive process. Particular attention is paid to apoptotic cell (neuronal) death, as well as, cell death and damage induced by secondary injury [2]. Central nervous system is considered as a radiosensitive system, and the degree of its dysfunction can be evaluated by electrophysiological, biochemical and behavioral parameters. Impairments of these parameters can be observed after local and total irradiation of the whole body [3].Recent studies revealed cranial radiation therapy impact on a wide range of brain functions resulting in cognitive and memory deficiency. Radiation-induced changes develop with a dose-volume-dependent severity.Highdoses of ionizing radiation induce reactive gliosis, white matter necrosis, vascular abnormalities, which are irreversible and result in clinical symptoms[4]. Low doses can also induce a wide array of cognitive dysfunctions without any significant morphological changes [5].Detrimental effects develop after months or years of brain irradiation. Acute, early delayed, and late injuries are observed [6]. Cognitive impairment is revealed in various degrees of learning difficulties, behavior changes, and memory deficits [7].The presence of cognitive disorders after exposure of high dose irradiation has a connectionto the hippocampus glial cells and proliferating progenitor cells in the subgranular zone of the dentate gyrus Radiation-induced cognitive dysfunctions is age-dependent,epidemiological studies revealed that the risk for cognitive disfunctions is higher during prenatal and childhood irradiation [13-14].

Populations of neural stem and progenitor cells located in the sub-granular zone of the dentate gyrus are radiosensitive. Radiation inhibits neurogenesis which results in hippocampal-dependent learning and memory impairment. Other mechanisms regulate the inhibition and/or recovery of neurogenesis and include a variety of stress-responsive of signaling mechanisms that impact the level of neuroinflammation [15].

MATERIALS AND METHODS

The experimental protocol was in accordance with the guidelines for care and use of laboratory animals as adopted by the Ethics Committee of the Tbilisi State Medical University (TSMU).

Animal care and maintenance.

Three month and one year old male mice (Mus musculus), were obtained from Vivarium of Tbilisi State Medical University. They were housed in animal cages, with room temperature maintained at 20^0-22^0C, relative humidity of 50-70% and an airflow rate of 15 exchange/h. Also, a time-controlled system provided 08:00-20:00 h light and 20:00-08:00 h dark cycles. All mice were given standard rodent chow diet and water from sanitized bottle fitted with stopper and sipper tubes.

Experimental design

After acclimatization for a week to laboratory conditions, the mice were divided into six different groups. The first controlgroup of three months old mice not irradiated, second group -experimental group of three months old irradiated mice, third control group of 1 year old mice and fourth experimental group – 1 year old irradiated mice, fifth experimental group – of18 months old mice and sixth group18 month old mice after 1 year of irradiation.Mice whole-body irradiation with ^{137}Cs was performed at a dose rate of 1,1Gy/min for the total dose of 5 Gy with a “Gamma-capsula-2”(group 2 and 4);

Spatial learning and formation of memory were estimated in the elevated-type multi-way maze.

The maze consists of 10 platforms (40x10 cm) fixed at height 25 cm. The motivation for movement along the maze under test conditions was to go back in the box-nest fixed at the end of the maze. Experiments were carried out seven days (five trials each day). Animals wereplaced in the start point facing the pathway of the maze.The familiarization session consistedof free exploration of the start and familiar arms for 10 min. On the first day, experimenter helped the animal to find the optimal way leading to box-nest. Number of errors(deviations from optimal trajectory) and total time for crossing the maze were calculated. Analysis of the obtained numerical data allowed us to estimate dynamics of learning process. Free passing in the labyrinth during 10-15 sec and the achievement of automated behavior was considered as a criterion of complete learning process.

All experimental areas werewiped with 20% ethanol after each trial. All behavioral experiments were conducted duringthe light cycleafter two hours of acclimation.

RESULTS AND DISCUSSION

Monitoring of spatial learning process of two animal groups in the elevated maze showed that animals of group I (control group of 3 month old mice;) when placed in the maze for the first time, needed the help of the experimenter only in two trials of the first testing day. Later they independently opened up the new environment and demonstrated research activity. On the 5thday mice of control group completely opened up spatial information. Others made not significant errors and the passage time significantly decreased. On the 6th and 7th days all mice of this group identified shortest way to the target and spent average 0.16 sec. At the end of the experiment, the majority of the animals could pass the maze in 2-3 sec.

Animals of group II (experimental group of 3month old mice) compared to control group showed restriction of movement. On the first day of experiment mice of this experimental group were not able to learn the way leading to the nest, even from the last platform. On second II-IV days mean number of errors decreased and mice reached the target-nest less than 3 minutes. Improvement of learning process and total mean time needed for crossing the maze was determined by middle part of the maze significantly increased the rate of the path recognition. Though, despite the visible improvement of spatial learning process V-VII days mean number of errors gradually increased and on the7thday almost approached the error number results of 1st day.Moreover, mean time of crossing the maze increased to 3.31 on 7th day of experiment. Despite the visible improvement in spatial learning process on II-IV days results obtained from control group animals differed significantly from the control group in both studied parameters (number of errors and time needed for crossing the maze) (Fig 1).

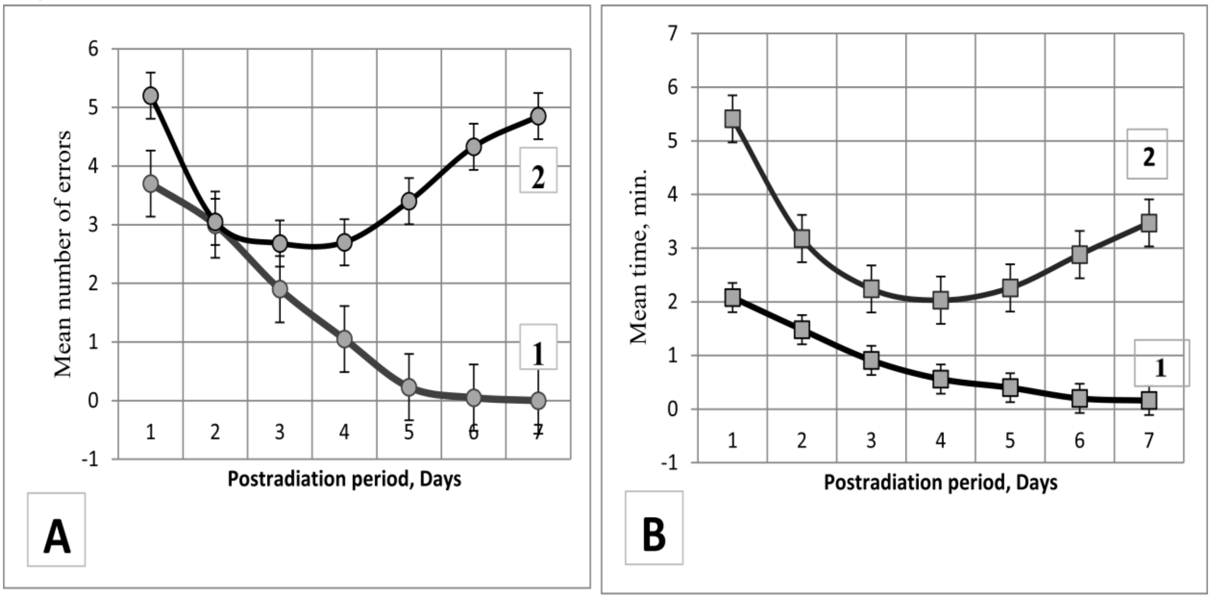

Fig.1 Effect of gamma-radiation on the cognitive parameters of 3 month old white mice (*During one week period*)

A-Mean number of errors in 3 month old mice;

B- Mean number of total time for crossing the maze (min).

The same test was carried out in 1 year old mice: Group III (control group of 1 year old mice), and Group IV (experimental group). in animals of control group number of mean errors and mean time for crossing the maze, accordingly, were equal to 3.85 and 2.04 was equal to 3.85. Later they independently opened up the new environment and number of errors decreased and on the 6thand 7thdays mice found the shortest way leading to target and spent average 0.19 sec.

Animals of group IV (experimental group of 1 year old mice) showed decreased number of errors and on the 5th day of experimentImprovement of learning process and total mean time was determined:mice reached the target-nest in 0.79 sec and number of errors was the same compared to control group.. On second II-IV days mean number of errors decreased and less than 3 minutes. Improvement of learning process and total mean time needed for crossing the maze was determined by middle part of the maze significantly increased the rate of the path recognition. Though, on VI-VII days mean number of errors and time gradually increased on 6th and 7th days of experiment (Fig 2).

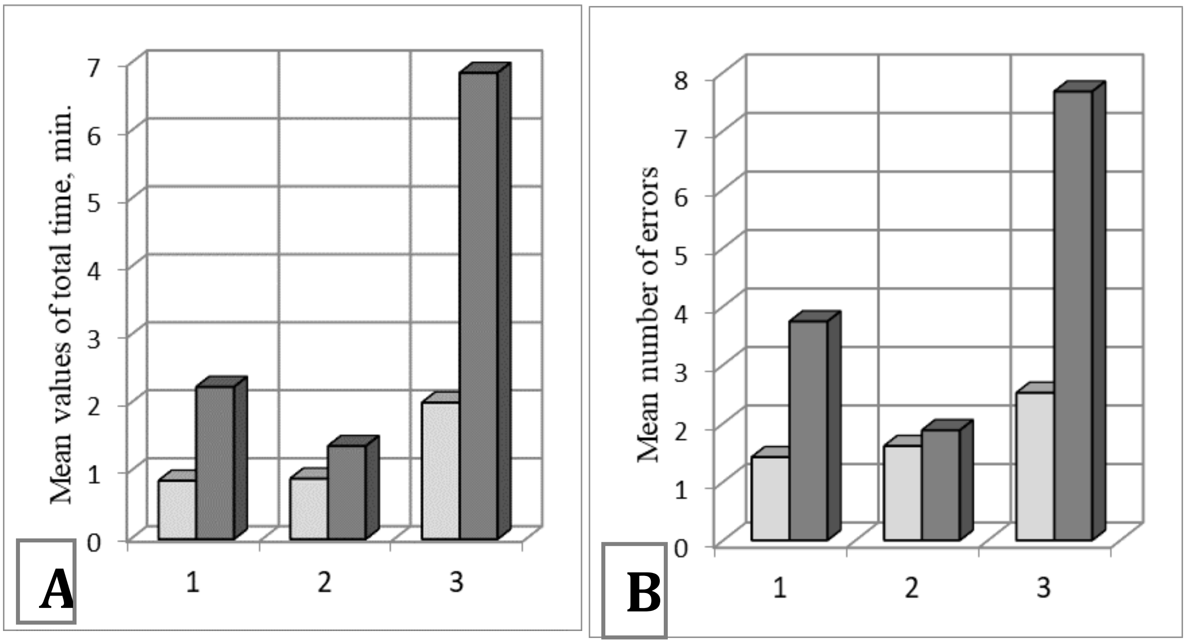

Fig.2. Effect of gamma-radiation on the cognitive parameters of 1 year old white mice (*During one week period*)

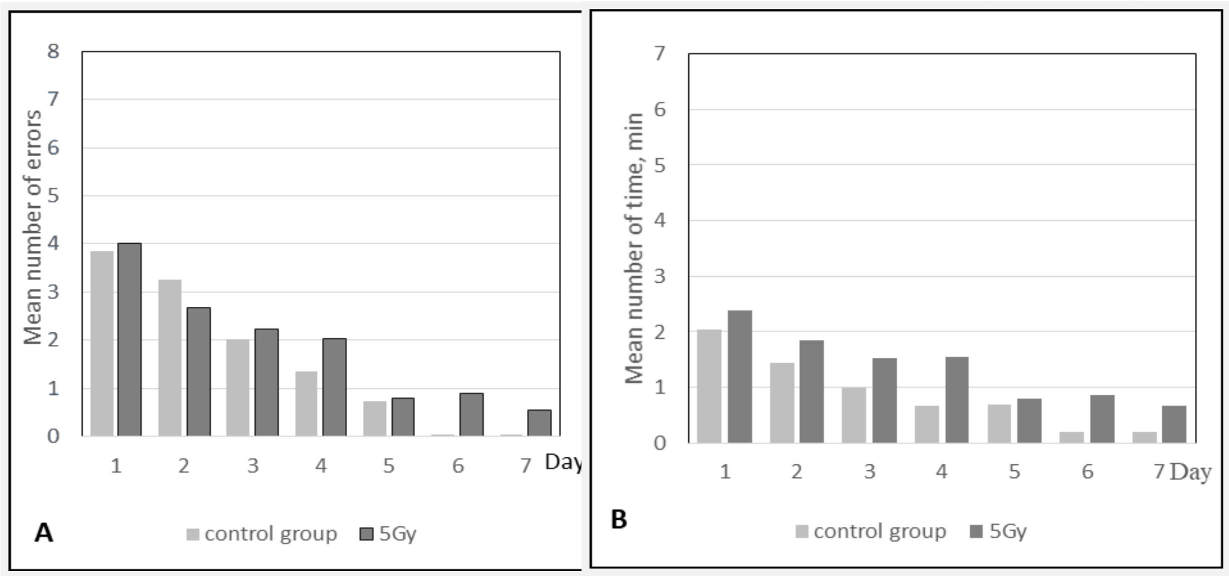

Fig.3 Effect of gamma-radiation on the spatial learning and memory of white mice

A- Effect of gamma-radiation on mean value of errors; **B-** Effect of gamma-radiation on mean number of time; 1-Changes after irradiation in 3 month old mice; 2- Changes after irradiation in 6 month old mice. 3- Changes after 1 year of irradiation.

In Sixth experimental group mean value of total time significantly increased in comparation to control group from 2.01 min to 6.86 min. The same results were obtained when comparing mean time of errors. After one year of irradiation spatial learning process significantly decreased in comparation to 3 and 6 monthold mice (Fig.3).

CONCLUSION

The results support that ionizing irradiation with total dose 5 Gy results in delayed spatial learning process in different age groups. Using a laboratory whitemouse model, we showed that ionizing radiation exposure causes spatial memory and behavior changes in different age groups of animals. Study of cognitive parameters reveled that gamma irradiation can be considered as a factor inducing radiation aging, what consolidates the contemporary evidence that radiation can accelerate aging and mortality.Dynamics of post-radiation effect formation can be divided intoshort and long-term effects. Age related radio resistance plays major role in the early stage of post-radiation recovery. Though, the main mechanism of late radiation effect formation can be related to radiation aging process.

REFERENCES

1. Kimeldorf D.J., Hunt E.L. (1965) Ionizing Radiation: Neural Function and Behavior. Academic Press, New York, 365 p.
2. Mickley G.A. (1987) Psychological effects of nuclear warfare In: J.J. Conklin, R.I. Walker (Eds.) Military Radiobiology. Academic Press, Inc., San Diego, p. 303–319.
3. Wong C.H., van der Kogel A.J. (2004) Mechanisms of radiation injury to the central nervous system: implications for neuroprotection. Mol. Interv., 4(5): 273–284
4. Gourmelon P., Marquette C., Agay D. et al. (2005) Involvement of the central nervous system in radiation-induced multi-organ dysfunction and/or failure. BJR Suppl., 27: 62–68.
5. Lawrence, Y.R., Li, X.A., el Naqa, I., Hahn, C.A., Marks, L.B., Merchant, T.E., Dicker, A.P., 2010. Radiation dose-volume effects in the brain. Int. J. Radiat. Oncol. Biol. Phys. 76,S20–S27.
6. Alison R. Preston, Howard Eichenbaum. Interplay of Hippocampus and Prefrontal Review Cortex in Memory. Current Biology VOLUME 23, ISSUE 17, PR764-R773, SEPTEMBER 09, 2013
7. Greene-Schloesser D, Robbins ME, Peiffer AM, Shaw EG, Wheeler KT, Chan MD. Radiation-induced brain injury: a review. Front Oncol (2012) 2:73
8. Katalin Lumniczky, Tünde Szatmári, and GézaSáfrány. Ionizing Radiation-Induced Immune and Inflammatory Reactions in the Brain, Front Immunol. 2017; 8: 517.
9. CalinaBetlazar,a,b Ryan J. Middleton,a Richard B. Banati,a,b, and Guo-Jun Liua,b,The impact of high and low dose ionising radiation on the central nervous system, Redox Biol. 2016 Oct; 9: 144–156.
10. Rola, R., Fishman, K., Baure, J., Rosi, S., Lamborn, K. R., Obenaus, A., Nelson, G. A. and Fike, J. R. Hippocampal Neurogenesis and Neuroinflammation after Cranial Irradiation with 56Fe Particles. Radiat. Res. 169, 626–632 (2008).
11. Michelle Monje 1, Moriah EThomason, Laura Rigolo, Yalin Wang, Deborah P Waber, Stephen E Sallan.
12. Alexandra J Golby Functional and structural differences in the hippocampus associated with memory deficits in adult survivors of acute lymphoblastic leukemia; Pediatr Blood Cancer 2013 Feb;60(2):293-300
13. Vladimir E. Zakhvataev - Possible scenarios of the influence of low-dose Ionizing radiation on neural functioning; Med Hypotheses 2015 Dec;85(6):723-35.
14. Rola R, Raber J, Rizk A, Otsuka S, VandenBerg SR, Morhardt DR, Fike JR. Radiation-induced impairment of hippocampal neurogenesis is associated with cognitive deficits in young mice, Experimental NeurologyVolume 188, Issue 2, August 2004, Pages 316-330.
15. Julie Constanzoa, Élora Midavaineb, JérémieFouquetc, Martin Lepagec, Maxime

Descoteauxd, Karyn Kirbyb, Luc Tremblayc, Laurence Masson-Côtéa, e, Sameh Gehaf, Michel Longpréb,BenoitPaquettea, Philippe Sarret, Brain irradiation leads to persistent neuroinflammation and long-termneurocognitive dysfunction in a region-specific manner, Progress inNeuropsychopharmacology& Biological Psychiatry.

16. Richard A. Britten, Leslie K Davis, Angela M Johnson, Sonia Keeney, Andrew Siegel, Larry D. Sanford, Sylvia J Singletary, GyörgyLonart. Low (20 cGy) Doses of 1 GeV/u 56Fe-Particle Radiation Lead to a Persistent Reduction in the Spatial Learning Ability of Rats. Radiation Research, 177(2):146-151 (2011).
17. Author links open overlay panelDanielaHladikSoileTapio, Effects of ionizing radiation on the mammalian brain, Mutation Research/Reviews in Mutation Research. Volume 770, Part B, October–December 2016, Pages 219-230.
18. M. Fouladi, E. Gilger, M. Kocak, D. Wallace, G. Buchanan, C. Reeves, N. Robbins, T. Merchant, L.E. Kun, R. Khan, A. Gajjar, R. Mulhern, Intellectual and functional outcome of children 3 years old or younger who have CNS malignancies, J. Clin. Oncol. 23 (2005) 7152–7160. [77] .
19. S. Fushiki, K. Matsushita, H. Yoshioka, W.J. Schull, InMunjal M. Acharya, Neal H. Patel,Brianna M. Craver,Katherine K. Tran,ErichGiedzinski, Bertrand P. Tseng,Vipan K. Parihar, Charles L. Limoli, Consequences of Low Dose Ionizing Radiation Exposure on the Hippocampal Microenvironment, Published: June 4, 2015
20. Hippocampal-related memory deficits and histological damageinduced by neonatal ionizing radiation exposure.Role of oxidative status.
21. Lucila Guadalupe Caceresa, Laura Aon Bertolinob, Gustavo Ezequiel Saracenob, María Aurelia ZorrillaZubiletea, Soledad Lucía Urana,Francisco Capanib, Laura Ruth Guelmana, Brain research, Volume 1312, 2 February 2010, Pages 67-78.