Form 21

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JINR Vice-Director

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SCIENTIFIC AND TECHNICAL VALIDATION OF THEME PROLONGATION AND INCLUSION IN THE 2015—2017 JINR TOPICAL PLAN

Theme code: 04-9-1077-2015/2017, Laboratory of Radiation Biology

Research area: (04) Condensed Matter Physics; Radiation and Radiobiological Research

Theme title: Research on the Biological Effect of Heavy Charged Particles with Different Energies

Theme leaders

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Abstract

Theme urgency. This Theme continues the studies completed within Theme 04-9-1077-2009/2017 "Research on the Biological Effect of Heavy Charged Particles with Different Energies." Throughout this period, the main aim was to study genetic disorders in cells of different origin and to do radiation physiology research. The investigations were focused on the regularities and mechanisms of molecular disorders in genetic structures of mammalian and human cells, formation of mutations of different types in lower and higher eukaryotes, and radiation damage to eye structures and central nervous system caused by ionizing radiations of different quality. In the course of radiation genetics research, studied in detail were regularities in the formation and repair kinetics of DNA double-strand breaks (DSBs) induced by accelerated boron and neon ions with an energy of 50 MeV/nucleon and high-energy carbon ions with an energy of 500 MeV/nucleon. It has been shown that in human cell nuclei there are sharp differences in the spatial distribution of damage induced by gamma rays and accelerated heavy ions. After gamma exposure, lesions in cells are distributed randomly; after heavy ion exposure,

they are localized along the ion tracks, thus forming "tracks" of clustered DNA DSBs. It has been established that the size and composition of the clusters are determined by physical characteristics of the acting radiation. In collaboration with specialists of the Institute of Biophysics of the Czech Academy of Sciences (Brno, Czechia), a research was performed on the kinetics of DNA damage induction and repair in normal and tumor cells after exposure to gamma rays, protons of different energies, and accelerated neon ions. Regularities were studied in the mutation process in mammalian cells for radiations in a wide range of linear energy transfer (LET) at different times after exposure. It has been found that the time of the maximal yield of mutant subclones depends on accelerated ions' LET. At higher LET, the mutant yield maximum shifts towards longer times. The LET dependence of the time of the mutant yield maximum is exponential, which can point to qualitative differences in genetic structure damage at different LET values that determine this shift.

As part of radiation physiology research, a large study cycle was completed to evaluate the dependence of the morphological and functional changes in the retina of small laboratory animals on the dose of irradiation with gamma rays, 170 MeV protons, and the genotoxic chemical agent methylnitrosourea. It has been shown that accelerated proton exposure at a dose of 25 Gy induces morphological changes in the retina and gives rise to the expression of inducible proteins associated with the apoptotic death of photoreceptor cells in the retina, which leads to the loss of retinal functional activity. The capacity has been revealed of the mature mouse retina for cellular and functional recovery and adaptive response. In collaboration with specialists of the Institute of Higher Nervous Activity and Neurophysiology of the Russian Academy of Sciences (RAS) and RAS Institute of Biomedical Problems, the action has been studied of accelerated carbon ions with an energy of 500 MeV/nucleon on the metabolism of the key neuromediators of the rodent brain. The most radiation-sensitive regions of the brain have been identified, where metabolism changed at early and late times after an exposure.

Along with experimental research, much theoretical work has been done on mathematical modeling of radiation-induced effects. Models have been developed of the mutation process induced by ultraviolet radiation in repair-deficient bacterial cells *E. cloli* and DNA DSB repair in higher organism cells after exposure to ionizing radiations of different quality. Within the proposed modeling framework, it is possible to describe DNA DSB formation and elimination kinetics. It has been shown that the approach can be used to model DNA DSB repair kinetics in repair-deficient cells.

A new concept of the radiation risk for manned interplanetary flights has been proposed and substantiated. The radiation risk for crew is connected, first of all, with the action of heavy nuclei of the galactic cosmic rays on the central nervous system structures. During the flight, this exposure can cause changes in the higher integrative functions of the brain and thus lead to disorders in crew's operational performance. The new paradigm calls for changes in the main fields of space radiobiology research and working out new radiation safety standards for manned deep space flights.

In view of the above, the solution of the mentioned fundamental and practical problems urgently requires that the regularities and mechanisms of the HCP effect be studied in detail at the molecular, cellular, tissue, and organismal levels of biological organization. Research on the molecular disorders in the genetic structures is important, first of all, for the analysis of the development of the most serious DNA damage: double-strand breaks (DSBs). An efficient

technique of clustered DNA DSB analysis designed and implemented at the LRB — the DNA foci method — will allow studying the induction of the most serious damage of the genetic apparatus by heavy ions and will make it possible to study the formation and repair of genetic damage both in proliferating tissues and in highly differentiated elements of the nervous system. The use of cells of different organisms in experiments (lower eukaryotes, mammals, and humans) will allow evaluation of the yield of gene and structural mutations induced by radiations in a wide LET range and studying formation of cytogenetic disorders for different doses of irradiation with charged particles of different energies. The approaches developed at the LRB to the problem of chromosome instability and studying mammalian and human cell response to exposure to different types of ionizing radiation at low doses will allow clearing up the mechanisms behind these reactions and evaluating the contribution of physicochemical processes (reactive oxygen species) and inducible repair mechanisms to their realization. Elucidating these fundamental cell processes as responses to exposure to charged particles of different energies can be the basis for understanding the tissue response of highly differentiated cell systems — the eye retina and CNS structures — to irradiation. In turn, these studies will allow the assessment of the system's integrity violation: cognitive and behavioral disorders. The practical orientation of this type of complex research for different activity areas is absolutely obvious.

The successful realization of the planned work will be based on JINR's unique stock of heavy ion accelerators. First of all, it is the Nuclotron, at whose beams it is possible to conduct a complex of studies at the molecular, cytogenetic, and organismal levels of biological organization; and the MC-400 cyclotron, which allows carrying out a wide range of molecular and cytogenetic research. The ion energies available at these accelerators overlap a significant part of the energy range of the GCR nuclei.

Main fields of research within the frameworks of the Theme:

- *Research on the regularities and mechanisms of molecular damage induction and repair in the DNA structure in mammalian and human cells for radiations with different linear energy transfer (LET)* in vivo *and* in vitro

Quantitative analysis of the regularities in the formation of different DNA lesions and their repair in the post-irradiation period is extremely important for clearing up the fundamental mechanisms of cell radiation responses: the lethal and mutagenic effects of radiation, apoptotic reactions, and radiation's transforming action. The methods of molecular disorder analysis in the DNA of different organisms' cells allow evaluating lesions not only in cell ensembles, but also in individual cells. Those are the DNA comet assay, DNA foci method, and different cytometric methods. The immunocytochemical DNA foci method allows not only identification of damage in individual cell nuclei, but also visualization of tracks of accelerated heavy ions passing through cell nuclei. Such an approach makes it possible to analyze the fine structure of DNA clustered damage and take into account the number of lesions along a charged particle track. The use of these methods will help answer the question: Do DNA damage distributions of different types influence the cells' repair ability, and if yes, then how? Also, it will be cleared up how different proteins detect clustered damage, how quick is identification of different types of DNA damage, and which proteins move first towards the DNA damage location.

- *Obtaining comparative data on the regularities in the induction of gene and structural mutations in mammalian and lower eukaryotic cells under exposure to sparsely and densely ionizing radiations with different LET.*

The problem of the mutagenic action of ionizing radiations of different quality — especially, accelerated heavy ions — on mammalian and human cells is still urgent and needs thorough research. In LRB's experiments on the irradiation of bacterial cells of different lines with accelerated heavy ions in a wide LET range, unique data were obtained that indicate a different character of the induction of gene and structural mutations. These differences are found in the character of the dose dependences (linear-quadratic for the gene mutations and linear for the structural mutations), which reflects the differences in the nature of the pre-mutation molecular damage and the mechanisms of the realization of the detected mutations. This research is extremely important for solving a number of practical tasks connected with standardization of radiation load on staff working in mixed fields of ionizing radiation, radiation safety measures for cosmonauts in long-term flights in deep space, and using charged particle beams in radiation therapy. In the course of this work, it is planned to determine the specifics of the mutagenic effect of sparsely and densely ionizing radiations on mammalian cells and evaluate the mutagenic effect of radiations of different quality. It is planned to continue studying the regularities in the induction of gene and structural mutations by heavy charged particles in a wide LET range on a model system of unicellular eukaryotes.

- *Research on the mechanisms of the heavy charged particle (HCP)-induced damage of the eye retina and its repair.*

By studying the effect of ionizing radiations on the molecular mechanisms controlling the CNS functions, work has been continued on the evaluation of radiation risk for planning long-term manned missions beyond the Earth's magnetosphere. The eye retina, which belongs to the CNS and was aptly defined by classic of neuroscience S. Ramón y Cajal as "part of the brain removed into periphery," is undoubtedly worth rigorous research. The studies pertain to the fundamental problem of damage and repair of terminally differentiated cells and tissues made up of them. Earlier results of LRB's research made it possible to single out the phenomenon of retina recovery from genotoxic exposure and show its stimulation by low radiation doses and its connection with retinal photoreceptor apoptosis. Further research suggests studying the mechanism of apoptosis decrease caused by radiation preconditioning of the retina and the possible participation of endogenous cell protectors and Müller glial cells in it. For this purpose, it is proposed to use the procedure of Müller cell visualization and detection in retinal microsections, which is based on the inclusion of the BrUdR proliferative marker in them. It is planned to evaluate the dependence of mouse retinal response on the radiation type and dose, which would allow describing the destructive character of radiation in correlation with functional changes in the retina.

- *Research on the character of the damage of central nervous system (CNS) cells and regularities of their death. Identification of the HCP-induced functional and morphological disorders in the CNS.*

Radiobiological research results give grounds to suggest that radiation-induced damage of CNS is one of the main risk components limiting the possibilities for the realization of missions beyond the Earth's magnetosphere. A high level of psychological stress and combined exposure to other negative factors (ionizing radiation, microgravity, abnormal environment, vibration, hypokinesia, etc.) predetermine an extremely high probability of CNS asthenia fraught with serious disorders of one's capacity for work — up to the development of symptoms that are typical of Alzheimer's disease. The main danger is associated with heavy ions of the galactic cosmic rays. To obtain adequate experimental data on the possible disorders in the cosmonauts' operational activity, it is planned to continue experiments on irradiated laboratory animals, study the neural and neurochemical mechanisms of the disorders, and extrapolate the results to humans. Experiments will be continued on the irradiation of primates, where operator's activity elements will be simulated, combined with studying the neurophysiological and neurochemical mechanisms behind these processes. It should be specially noted that in monkeys, like in humans, of tremendous significance are individual specifics of the higher nervous activity and regulation, which largely determine individual resistance to different stress factors, including radiation exposure.

- *Mathematical modeling of the effects of ionizing radiations with different LET at the molecular and cellular levels. Development and analysis of mathematical models of the molecular mechanisms of ionizing radiation-induced disorders in the CNS structure and functions.*

Mathematical modeling of radiation-induced effects is a dynamically developing field of modern radiobiology. Significant success has been achieved in the development of mathematical models describing different stages of the radiation damage of living systems — from lesions at the individual molecule level to changes in the functioning of some physiological systems. Modern research in quantitative radiobiology is largely concerned with primary DNA damage induced by ionizing radiations of different quality and description of DNA repair processes. The continuing accumulation of experimental data on the molecular mechanisms of DNA repair makes for the development of quite detailed models characterizing radiation-induced damage repair. A topical issue is the mathematical modeling of some aspects of radiation action on the CNS structure and functions, which can show up as disorders in signal transfer, ion regulation, synapse functioning, gene expression, protein synthesis, and neurogenesis in hippocampus. The remote consequences of radiation exposure include disorders in cognitive functions like memory and spatial orientation. To solve the stated problems, for the first time a coordinated use is proposed of a set of computational methods established in modeling charged particle transport through matter, radiation chemistry, molecular dynamics, polymer biophysics, genetic regulatory networks, and information processing and transmission in neural networks.

- *Calculation of shielding for new nuclear physics facilities, evaluation of the radiation environment, and development of radiation safety systems*.

Work will be continued on the development of heavy ion beam dosimetry methods as applied to radiobiological research at charged particle accelerators, resolving the radiation safety issues at the NICA complex, and the development of neutron spectrometry and radiometry techniques;

participation in nuclear planetary science and astrobiology research will be continued. Annually, two radiobiological experiments are planned to be conducted at the Nuclotron's heavy nuclear beams and two at the MC400 cyclotron nuclear beams. Besides, periodic irradiation of biological objects at the Phasotron's medical beam and the Rocus-M gamma therapeutic facility is planned.

Theme stages

1. Radiobiological research at charged particle beams

   Leader: *E.A. Krasavin* + 43 positions

2. Radiation research

   Leader: *G.N. Timoshenko* + 12 positions

3. Photoradiobiological research

   Leader: *M.A. Ostrovsky* + 4 positions

4. Research on the regularities and mechanisms of heavy charged particle-induced structural and functional disorders in the central nervous system.

   Leaders: *A.A. Ivanov* + 7 positions

5. Mathematical modeling of radiation-induced effects of ionizing radiations

   Leader: *A.N. Bugay* + 7 positions

6. Educating specialists in radiation protection and radiobiology

   Leaders: *E.A. Krasavin, S.Z. Pakuliak*

Results expected upon the Theme completion

The work planned within the framework of the Theme is continuation of research started at the LRB earlier. The following results are expected fields:

Research on the regularities and mechanisms of molecular damage induction and repair in the DNA structure in mammalian and human cells for radiations with different linear energy transfer (LET) in vivo *and* in vitro:

- to determine regularities in the induction of clustered DNA double-strand breaks (DSBs) by accelerated heavy ions in human skin fibroblast nuclei and radioresistant U87 tumor cells;
- to study the kinetics of clustered DNA DSB repair in the post-irradiation period in human skin fibroblast nuclei and radioresistant U87 tumor cells;
- to study the formation and repair kinetics of clustered DNA DSBs in the post-irradiation period after accelerated heavy ion exposure of neuron precursor cells, adult neurons, and

glial cells of the mammalian central nervous system (CNS) using cell subpopulation markers NeuN, doublecortin, GFAP, BrdU, and calbindin;

- to determine regularities in the induction of different types of DNA damage (single-strand breaks, base damage, and complex DNA damage) by heavy charged particles (HCP) in human fibroblast nuclei;
- to evaluate the proportion of different DNA DSB repair pathways in human fibroblasts after exposure to radiations of different quality by immunocytochemical staining of repair proteins RAD51 (HR) and DNA PKcs (NHEJ);
- to study the expression of genes encoding the proteins and caspases which participate in repair (RAD51, DNA PKcs, NBS1, MRE11, etc.) in human fibroblasts after HCP exposure;
- to determine regularities in apoptosis induction in human skin fibroblasts and mammalian CNS neurons by accelerated heavy ions;
- to study the expression of genes encoding proteins and caspases which participate in apoptosis induction in human fibroblasts and nervous cells after exposure to charged particles of different energies;
- to determine regularities in DNA DSB formation and repair in cancer and normal cells from near the tumor taken from radiation therapy patients;
- to determine regularities in DNA damage formation and elimination *in vivo* and *in vitro* in mammalian CNS neurons after exposure to gamma rays and accelerated heavy ions;
- to study molecular disorders in hippocampus and cerebellum at different times (up to three months) after HCP exposure;
- to study the effect of age-related changes in the CNS on damage induction and repair in mammalian neurons after exposure to ionizing radiations of different quality;
- to determine regularities in DNA DSB induction and repair kinetics in mammalian CNS neurons and peripheral blood lymphocytes in the presence of immunomodulators after exposure to ionizing radiations of different quality.

Obtaining comparative data on the regularities in the induction of gene and structural mutations in mammalian and lower eukaryotic cells after exposure to sparsely and densely ionizing radiations with different LET:

- to do a cytogenetic and molecular analysis of the obtained mutant subclones;
- to study chromosome and genome instability in irradiated cell descendants ;
- to complete research on the action of standard exposure gamma rays on different genetic systems of yeasts that allow studying all mutation event types;
- to study the influence of the mitochondrial genome as an oxidative stress source on the mutagenic and lethal action of radiation using the rho^- and rho^0 mutations of the yeast mitochondrial genome;

- to refine a technique of the quantitative evaluation of the reactive oxygen species (ROS) level in yeast cells using fluorescent staining;
- using a microplate reader Synergy H1m (BioTek Instruments, Inc.) , to measure the ROS level in yeast cells after gamma ray and heavy ion exposure.

Research on the mechanisms of the heavy charged particle (HCP)-induced damage of the eye's retina and its repair:

- to determine disorders in retinal cell elements, first if all in Müller glial cells and photoreceptor cells, induced by gamma rays, accelerated protons, and accelerated heavy charged particles;
- to study functional disorders of the retina by recording its electrical activity after exposure of mouse eyes to gamma rays, accelerated protons, and accelerated heavy charged particles;
- to evaluate the retinal ability to recover after a fractioned radiation exposure.

Research on the character of the damage of central nervous system (CNS) cells and regularities of their death. Identification of the HCP-induced functional and morphological disorders in the CNS:

- in experiments on rodents, to study quantitative regularities in the development of morphofunctional disorders in the CNS induced by corpuscular radiation: to obtain the effect dependence on the dose and time;
- to compare the effects of sparsely and densely ionizing radiations on the CNS;
- to evaluate the contribution of the metabolic changes developing in the irradiated organism on CNS functioning;
- to study pharmacological effects of nootropic drugs on CNS functioning after a corpuscular exposure;
- to evaluate the effect of traditional radioprotective drugs on CNS functioning for corpuscular radiation;
- to study radiation exposure action on the exchange of monoamines and their metabolites in different brain structures actively involved in behavior realization and motor activity and forming the emotional and motivational states (neocortex, hippocampus, hypothalamus, nucleus accumbens, and prefrontal cortex);
- to study the level of the apoptotic death of neurons in different parts of the rodent brain at different times after ionizing radiation exposure (by measuring the caspase-3 level);
- to study the action of radiations of different quality on neurogenesis and neuron growth and development: in particular, to evaluate the level of the brain's neurotrophic factor BDNF and glial cells' neurotrophic factor GDNF, which play an important role in the proliferation, differentiation, and development of neurons — in particular, in the

hippocampus; and to estimate the level of the nerve growth factor in different parts of the brain;

- to do a morphofunctional research on the delayed effects of exposure to ionizing radiations of different quality;
- to compare the results of gamma, high-energy proton, and accelerated heavy ion exposures;
- to correlate the changes observed at the molecular mechanism levels with behavioral test results.

Mathematical modeling of the effects of ionizing radiations with different LET at the molecular and cellular levels. Development and analysis of mathematical models of the molecular mechanisms of ionizing radiation-induced disorders in the CNS structure and functions:

- to perform mathematical modeling of induction and repair mechanisms of the main DNA damage types in mammalian and human cells;
- to work out a mathematical model of the development of radiation-induced oxidative stress in nervous cells;
- to develop methods of the theoretical calculation of radiation-induced damage in the sensitive structures of nervous cells: the membrane, cytoskeleton, ion channels, and synaptic contacts;
- to develop mathematical models of brain neural networks and, based on them, to do a theoretical evaluation of radiation-induced cognitive function disorders.

Calculation of shielding for new nuclear physics facilities, evaluation of the radiation environment, and development of radiation safety systems:

- to provide physics support for the radiobiological experiments at JINR's charged particle accelerators;
- to continue development of methods of evaluating heavy ion beam performances and beam dosimetry;
- to continue the Laboratory's participation in designing JINR's new nuclear physics facilities as regards radiation safety (first of all, the NICA accelerator complex);
- to continue the development of neutron spectroscopy methods for JINR's nuclear physics facilities and their environment;
- to provide operation of the DAN experimental stand and to continue the Laboratory's participation in designing, testing, and calibration of nuclear planetary science tools for studying the elemental composition of the Solar System's celestial bodies and search for water ice.

Participating organizations

Within the framework of the Theme, the LRB will be cooperating with the following scientific institutions of JINR Member States and other countries: Armenia (), Belarus (), Bulgaria (IE BAS, NCRRP), Czechia (IBR ASCR, CTU, NRI), Italy (UNIUD), Moldova (), Mongolia (NUM), Poland (SU), Russia (IBMP RAS, SIIF RAMS, MSU, ITEP, IHNAN RAS, IP), Romania (UMF, UAIC, IBR), Slovakia (CU), and South Africa (iThemba Labs).

Duration of work performance: 2018 — 2020

Full estimated cost of Theme realization according to JINR's Seven-Year Plan:

Year	thousand USD
2018	245
2019	254.5
2020	266
Total	765.5

Other financing sources: no

Endorsed:

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