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

Project Abstract

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.

The following main fields of research are planned 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*
- Obtaining comparative data on the regularities in the induction of gene and structural mutations in mammalian and lower eukaryote cells under exposure to sparsely and densely ionizing radiations with different LET.
- Research on the mechanisms of the heavy charged particle (HCP)-induced damage of the eye retina and its repair.
- 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.
- 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.
- Calculation of shielding for new nuclear physics facilities, evaluation of the radiation environment, and development of radiation safety systems.

Results expected in three years

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 of 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.

Form 26

Schedule proposal and resources required for the fulfillment of the Project

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

Facility units and systems; resources; financing sources			Cost of the facility units, thousand USD. Resource requirements, hours	Proposals of the participating Laboratories on the distribition of finances and resources		
				2018	2019	2020
Necessary resources	Standard hour	Nuclotron	144 h	48 h	48 h	48 h
		MC400 cyclotron	288 h	96 h	96 h	96 h
		Rocus-M	300 h	100 h	100 h	100 h
		Phasotron's medical beam	102 h	34 h	34 h	34 h
Financing source	Budget	Theme 1077	1135	316	374	445

Project leaders

E.A. Krasavin

G.N. Timoshenko

Staff resources

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*

Work schedule

№	Main work and stages	Work periods (year, quarter)	
		Start	End
1	Finding regularities in the formation of HCP-induced clustered DNA DSBs in human skin fibroblast nuclei and radioresistant tumor cells U87	2018, I	2020, IV
2	Studying the repair kinetics of HCP-induced clustered DNA DSBs in human skin fibroblast nuclei and radioresistant tumor cells U87	2018, I	2020, IV
3	Studying regularities in the induction of different types of DNA damage (single-strand breaks, base lesions, complex damage) by HCP in human fibroblast nuclei	2018, I	2020, IV

4	Evaluation of the proportion of different DNA DSB repair pathways in human fibroblasts after exposure to ionizing radiations of different quality — using immunocytochemical staining of the repair proteins RAD51 (HR) and DNA PKcs (NHEJ)	2018, III	2020, IV
5	Studying regularities in the formation and kinetics of the repair of clustered DNA DSBs after HCP exposure in precursor cell nuclei and mature neurons as well as in mammalian glial CNS cells — using the cell subpopulation markers NeuN, doublecortin, GFAP, BrdU, and calbindin.	2018, I	2020, IV
6	Experiments on the expression of the genes that code the proteins participating in HCP-induced damage repair (RAD51, DNA PKcs, NBS1, MRE11, etc.) in human fibroblasts.	2019, I	2020, IV
7	Studying regularities in apoptosis induction in human skin fibroblasts and mammalian CNS neurons after HCP exposure	2018, I	2020, IV
8	Experiments on the expression of the genes encoding the proteins and caspases involved in apoptosis induction in human fibroblasts and nervous cells after HCP exposure	2019, I	2020, IV
9	Studying regularities in DNA DSB formation and repair in cancer cells and normal cells near tumors from patients undergoing radiation therapy	2018, I	2020, IV
10	Studying regularities in DNA DSB formation and elimination in rat hippocampus cells *in vitro* using a primary hippocampus culture from P0—P1 age rats	2018, I	2020, IV
11	Evaluation of regularities in DNA DSB formation in mammalian CNS neurons after gamma ray and accelerated heavy ion exposure	2018, I	2019, IV
12	Studying DNA DSB repair kinetics in mammalian CNS neurons after gamma ray and accelerated heavy ion exposure	2018, I	2019, IV
13	Studying regularities in the formation of clustered DNA DSBs in mammalian CNS neurons after HCP exposure	2018, I	2020, IV
14	Studying clustered DNA DSB repair kinetics in mammalian CNS neurons after HCP exposure	2018, I	2020, IV
15	Studying the effect of age-related changes in the CNS on damage induction in mammalian brain neurons after		

	exposure to ionizing radiations of different quality	2018, I	2020, IV
16	Studying the effect of age-related changes in the CNS on DNA DSB repair kinetics in mammalian brain neurons after exposure to ionizing radiations of different quality	2018, I	2020, IV
17	Studying regularities in DNA DSB induction and repair in mammalian CNS neurons and peripheral blood lymphocytes after exposure to ionizing radiations of different quality in the presence of immunomodulators	2018, I	2019, IV
18	Experiments to study the action of gamma-rays, protons, and HCP on normal and tumor human cells (lymphocytes and Cal51 carcinoma cells, respectively) by the classical metaphase method and FISH-method.	2018, I	2020, IV
19	Research on the mutagenic effect of sparsely and densely ionizing radiations on mammalian cells (V-79) in the remote period after irradiation. Molecular and cytogenetic analysis of the genome instability of mutant subclones.	2018, I	2020, IV
20	Identification of regularities in the induction of gene and structural mutations in lower eukaryote cells under exposure to radiations in a wide LET range.	2018, I	2020, IV
21	Experiments on studying disorders in retinal cell elements — first of all, in Müller glial cells and photoreceptor cells — after mouse eye exposure to gamma rays, accelerated protons, and accelerated heavy charged particles	2018, I	2020, IV
22	Studying functional disorders of the retina by its electrical activity analysis after mouse eye exposure to gamma rays, accelerated protons, and accelerated heavy charged particles	2018, I	2020, IV
23	Studying the retinal capacity for recovery after fractioned radiation exposure	2018, I	2020, IV
24	Evaluation of the ROS and RNS protein kinase level in SIM-A9 microglial cells after exposure to gamma rays, protons, and heavy charged particles	2018, I	2019, II
25	Evaluation of inflammation cytokine yield in SIM-A9 microglial cells after exposure to gamma rays, protons, and heavy charged particles	2019, III	2020, IV
26	Putting into operation an upgraded high-efficiency liquid chromatography facility with electrochemical detection	2018, I	2019, I

27	Studying the action of ionizing radiations of different quality on the metabolism of the main excitatory (dopamine, histamine, acetylcholine, glutamate) and inhibitory (GABA) neurotransmitters in different morphological structures of the rat and mouse brain	2018, I	2020, IV
28	Evaluation of anxiety, depressive behavior, emotional responsiveness, habituation, and operational and spatial memory-based learning ability in rats and mice after exposure to ionizing radiations with different physical characteristics	2018, I	2020, IV
29	Improvement of the fluorometric method of caspase-3 (apoptotic cell death marker) activity evaluation in tissues of different structures of the rat and mouse brain	2018, I	2020, IV
30	Evaluation of the connection between changes in the performance of the main neurotransmitter systems and apoptotic neuron death after exposure	2018, I	2020, IV
31	Studying radiation-induced changes in the functioning of some receptor groups (opiate, dopamine, histamine, acetylcholine, glutamate, and GABA)	2019, II	2020, IV
32	Mathematical modeling of induction and repair of the key DNA damage types after HCP exposure	2018, I	2019, IV
33	Mathematical modeling of the development of radiation-induced oxidative stress in nervous cells	2018, I	2019, IV
34	Computer modeling of the formation of radiation-induced damage in the cell membrane and cytoskeleton	2018, I	2019, IV
35	Computer modeling of the formation of radiation-induced damage in membrane ion channels and synaptic contacts	2018, I	2019, IV
36	Modeling the electrical activity of neurons of different types and morphology after radiation damage	2018, I	2020, IV
37	Modeling neural networks' electrical activity in the prefrontal cortex after radiation damage	2019, I	2020, IV
38	Modeling neural networks' electrical activity in the hippocampus after radiation damage	2019, I	2020, IV
39	Development of mathematical models for the prediction of radiation-induced cognitive function disorders	2020, I	2020, IV
40	Physical support of the radiobiological experiments at JINR's charged particle accelerators.	2018, I	2020, IV

41	Development of methods of evaluating heavy ion beam performances and beam dosimetry.	2018, I	2020, IV
42	Development, tests, and calibration of nuclear planetary science instruments	2018, I	2020, IV
43	Designing JINR's new nuclear physics facilities as regards radiation safety.	2018, I	2020, IV

Project Leaders

E.A. Krasavin

G.N. Timoshenko

Form 29

Estimate of expenditures for the Project

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

	Expense item	Full cost, thousand USD	1st year	2nd year	3rd year
	Direct expenses for the Project				
1.	Materials	429.5	136.5	142.2	151.2
2.	Equipment	185.6	58.5	62.3	64.8
3.	Payments for agreement-based research	-	-	-	-
4.	Travel allowance, including:	150	50	50	50
	а) non-rouble zone countries				
	б) rouble zone countries				
	в) protocol-based				
	Total direct expenses	765.5	245	254.5	266

Project Leaders

E.A. Krasavin

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Director Laboratory of Radiation Biology

E.A. Krasavin

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