Form 21

APPROVED

JINR Vice Director

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“____“_____2020

SCIENTIFIC AND TECHNOLOGICAL RATIONALE FOR EXTENDING A THEME

to be included in the

TOPICAL PLAN FOR JINR RESEARCH 2021–2023

Theme code 04-9-1077-2009/2023 **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 OF DIFFERENT ENERGIES

Theme leaders: **E.A. Krasavin**

A.N. Bugay

Abstract

Accelerated heavy charged particles are a powerful tool for addressing fundamental issues of modern radiobiology and genetics. Evaluation of their biological effectiveness is essential for solving radiation medicine problems. As is known, radiation therapy with proton and carbon ion beams is one of the most efficient ways of treating hard-to-reach malignant neoplasms — in particular, brain tumors. Besides, high-energy protons and heavy ions are the largest component of space radiation and would present the highest radiation risk to the crews of the manned missions beyond Earth's magnetosphere. In this connection, particle beam therapy of tumors and ensuring the radiation safety of the future manned interplanetary flights are the top priorities of modern radiobiology.

During the previous stage of the Theme «Research on the biological effect of heavy charged particles of different energies» (04-9-1077-2009/2023), with the use of JINR's accelerators, a number of principal issues have been resolved concerning the mechanisms of the biological action of accelerated charged particles in a wide range of linear energy transfer (LET). Research has been focused mainly on genetic damage to cells of different origin and radiation-induced physiological disorders in mammalian organisms.

In *radiation genetics* experiments at JINR's basic facilities, formation and repair kinetics of DNA double-strand breaks (DSBs) induced by accelerated heavy ions has been studied in detail. Dose dependences of clustered DNA damage formation frequency in human cells have been obtained. Accelerated heavy charged particles have been found to be highly efficient in inducing clustered damage. It has been shown that the structure, size, and shape of clustered damage depend on ions' linear energy transfer (LET). It has been found that after accelerated multi-charged ion exposure, the elimination kinetics of radiation-induced genetic damage in cells is slower than after γ-ray exposure, which points to a decrease in DNA DSB repair efficiency. A series of works entitled "Research on molecular damage formation in genetic structures of human and mammalian cells after exposure to low and intermediate-energy accelerated heavy ions" won JINR's First Prize in 2020. Along with molecular aspects of the biological action of accelerated heavy ions, the mutation process has been studied in mammalian cells. In a wide LET range, the mutant clone yield has been studied at different times after exposure. It has been established that in the post-irradiation period the highest mutant clone yield depends on particles' LET and post-irradiation time.

In *radiation physiology research*, morphological and functional changes have been studied in the retina and different parts of the brain in rodents. It has been found that the retina is highly radioresistant as evaluated based on morphological and functional damage in

the post-irradiation period. Quantitative regularities have been established in the development of morphofunctional disorders in different brain parts after accelerated proton and carbon ion exposure; pharmacological action of nootropic drugs on radiation exposure effects has been evaluated (in 2018, patent for an invention No. 2666937 was received). Behavioral disorders in exposed animals have been studied with different test systems in the post-irradiation period. In cooperation 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 of high-energy (500 MeV/nucleon) carbon ions on the metabolism of the key neuromediators of the rodent brain has been evaluated. The most radiation-sensitive parts of the brain have been identified, where metabolism changed at shorter and longer times after exposure.

A new *concept of the radiation risk for manned interplanetary flights* has been proposed and justified. The concept links the radiation risk to cosmonauts mainly to the action of the heavy nuclei of galactic cosmic rays on the central nervous systems' structures. During the flight, this exposure can affect the brain's highest integrative functions and result in the disruption of the crew members' operator activity. The new paradigm requires a change of the main fields of space radiobiology research and indicates the necessity of the development of new regulations on the radiation safety of the manned deep space flights.

Along with experimental research, a significant amount of theoretical work has been done on the *mathematical modeling of radiation-induced effects*. Methods have been developed of calculating the formation of DNA damage of different types in charged particle tracks passing through brain cells and structures. A molecular dynamics approach has been proposed to the quantitative account of the action of mutations in hippocampal neurons' genes on the condition of synaptic receptors. The influence has been modeled of radiation-induced effects on the functioning of the brain's neural networks.

Molecular radiobiology research on the effect of some modifiers on the DNA DSB yield in the case of exposure of human normal and tumor cells to radiation in a wide LET range has shown that in the presence of some drugs the yield of DNA DSBs — the damage that leads to cell death in the post-irradiation period — is modified to different degrees. These modifiers include the officinal drugs 1-β-D-arabinofuranosyl cytosine (Ara-C) and hydroxyurea (HU). In the presence of these modifying agents, after γ-ray and accelerated proton exposure, the DNA DSB yield significantly increased during post-irradiation incubation of cells. After high-LET heavy ion exposure, the radiomodifiers' influence sharply weakened. Research on the mechanism of these agents' amplifying effect on cells'

radiosensitivity has shown that Ara-C is kind of a Trojan horse at the molecular level. With the use of immunocytochemical methods, it has been found that, in the presence of the modifiers, after cell exposure to accelerated protons, the DNA DSB yield increases, and cells' radiosensitivity sharply rises to the level that is observed in the case of carbon ion beam exposure. An increase in the DNA DSB yield in the presence of Ara-C and HU is explained by an increase in the yield of enzymatic DNA DSBs which developed from single-strand breaks. Using the discovered phenomenon can be considered an efficient approach to improving *radiation therapy* techniques (in 2019, patent for an invention No. 2699670 was received).

In view of the above, solving the related fundamental and practical problems urgently requires the continuation of detailed research on the mechanisms of the action of accelerated heavy charged particles at the molecular, cellular, tissue, and organismal levels of biological organization. Studying molecular damage to genetic structures is important, first of all, for the analysis of the induction of the most severe DNA lesions: double-strand breaks (DSBs). An efficient method of the three-dimensional analysis of clustered DNA DSBs (the DNA foci method), which has been developed and introduced at the LRB, will allow studying the formation of the heaviest damage to the genetic apparatus caused by exposure to accelerated multi-charged ions and make it possible to conduct research on the formation and repair of genetic damage in proliferating tissues and in highly differentiated elements of the nervous system. Finding out the mechanisms of response to the action of accelerated charged particles of different energies will lay the ground for understanding the tissue reactions of highly differentiated cell systems — structures of different parts of the CNS — to radiation exposure. In turn, this research will allow evaluating CNS integrity violations: disorders of cognitive functions and behavior. The practical character of such complex research is absolutely clear from the point of view of a number of applied areas — first of all, solving the problems of human space radiobiology.

During the conduction of the planned research, of special importance will be finding out the mechanisms behind increasing the biological effectiveness of proton and photon beams on radioresistant tumor cells and studying radiation action on tumors transplanted into experimental animals. Earlier results on the modifying effect of agents like cytosine arabinoside in combination with other drugs on DNA DSB yield for ionizing radiations of different quality, along with the possible prospects for the clinical use of DNA synthesis inhibitors of this type and ionizing radiation, point to the necessity of further research within the scope of the proposed Theme.

Main fields of research within the scope of the Theme

- *Molecular radiobiology*

For a number of years, using different techniques, the Laboratory of Radiation Biology (LRB) has been conducting research on the molecular damage induced by electromagnetic and particle radiation in the genetic structures of mammalian and human cells. For these investigations, immunocytogenetic and immunohistochemical techniques have been widely used in recent years. These methods allow not only the quantitative analysis of genetic disorder formation, but also taking into account the spatial distribution of damage in cells' genetic structures.

Finding out how different proteins detect clustered damage, how fast the identification of different DNA damage types is, and which proteins are the first to reach the DNA damage location is essential for solving many fundamental problems of cytology and genetics. DNA DSB induction in certain parts of the cell's genome causes a specific phosphorylation of the H2AX histone in chromatin surrounding the lesion, which shows up as the formation of the so-called γH2AX foci. The internal structure of these radiation-induced foci (RIF) is a network of biochemical pathways triggered by cells in response to the appearance of a DNA lesion to restore DNA integrity. The planned studies of RIF nanostructure using fluorescent and confocal microscopy and single molecule localization microscopy — high-resolution nanoscopy — will allow finding out how radiation's physical characteristics and chromatin structure at a local DNA DSB formation site affect RIF micro-and nanostructure and, on the other hand, how the micro- and nanostructure of damaged chromatin and RIF affects the choice of a damaged site repair pathway and, further, repair kinetics and efficiency.

Of special interest is the comparative analysis of the patterns and mechanisms of the induction and repair of DNA molecule damage in normal cells and radioresistant tumor cells exposed to γ-rays, protons, and heavy ions of different energies. Using the immunocytochemical method with different RIF formation markers (H2AX, 53BP1, Rad51, etc.) will allow the analysis of the chromatin structure on a damaged site, its change kinetics, and determining which repair type makes the main contribution to damage elimination. On the basis of these data, the regulation mechanisms will be identified that determine the repair pathway choice depending on the chromatin structure on a local site, cell type, cell cycle phase, and ionizing radiation's physical characteristics.

- *Radiation genetics*

The mutagenic action of ionizing radiation of different quality — especially, accelerated heavy ions — on mammalian and human cells have still been poorly studied. It is planned to continue the research that has already been underway at the LRB on the efficiency of the induction of different types of gene and structural mutations depending on the radiation's dose and LET, repair status, and oxidative stress development, as well as to clarify the mechanisms behind genetic stability.

Haploid tester strains of the yeast *Saccharomyces cerevisiae* are used to detect different molecular events, including base pair changes, omissions of one nucleotide, deletions, and recombinational rearrangements. It is planned to continue research on the mutagenic action of sparsely and densely ionizing radiations using these tester systems; to determine more precisely mutations' molecular nature, induced point mutation sequencing and the electrophoretic and restriction analysis of deletion mutants are going to be used.

Besides nuclear DNA, there is mitochondrial DNA (mtDNA) in cells. The latter's mutagenesis and functional importance are still poorly studied. It is planned to investigate the development of mitochondrial mutations and the influence of mitochondrial respiration-deficient mutations on the lethal and mutagenic action of radiation.

Concerning repair and mutagenesis, of extreme importance is nucleotide balance. Human inosine triphosphate pyrophosphohydrolase hITPA ensures the balance of the quantity of non-canonical nucleotides in the cell. It is planned to investigate the mechanism of phosphatase activity regulation and the influence of the enzyme's inactivation on radiosensitivity, cell cycle regulation at the checkpoints, apoptosis, and ageing.

In the course of research on radiation-induced mutagenesis in V79 Chinese hamster cells, genome instability has been discovered in the HPRT mutants throughout numerous generations of a mutant cell. The analysis of structural damage in the *hprt* gene in descendants of a mutant cell will allow understanding possible mechanisms behind genome instability.

- *Radiation cytogenetics*

Chromosomal aberrations have been studied for more than half a century, but there are still many unanswered questions in this area. The state-of-the-art technique of multicolor (or multiplex) fluorescent in situ hybridization (mFISH), which is now used at the LRB, allows identifying each pair of human and animal chromosomes. The main advantage of this method

is that it is possible to study complex chromosomal aberrations (three and more breaks in two and more chromosomes).

Using mFISH will provide new insights into the mechanisms of radiation-induced aberration formation. In radiation cytogenetics, the induction of complex chromosomal aberrations by accelerated protons and intermediate energy heavy ions (tens of MeV/nucleon) has still been poorly studied. It is planned to conduct research on the action of such particles on human and mammalian normal and tumor cells.

The mFISH method is also promising for studying the long-term consequences of the radiation exposure of the organism. It is planned to study in experiments on laboratory animals the residual chromosomal aberration yield in bone marrow cells and blood lymphocytes during 6–8 months after exposure by both mFISH and the standard metaphase method; in parallel, the radiation-induced response of the hematopoietic, immune, and other regulatory systems of the organism will be studied, which is very important for solving problems of space radiobiology and radiation oncology.

- *Radiation physiology*

In the coming period, radiation physiology research will be focused mainly on behavioral reaction disorders in irradiated animals and pathomorphological changes in different structures of the brain, spinal cord, and critical organs and systems of rodents. The evaluation of behavioral reactions is going to be made using the whole set of modern zoopsychology techniques and equipment, including test systems for assessing long-term and short-term memory, emotional reactivity, the anxiety level, and motor reflexes. Behavior parameters will be analyzed with modern video tracking tools. To study pathologies in the animal organism caused by radiation exposure, it will be necessary to use delicate techniques of surgical intervention and catheterization. With this purpose, the research program includes the introduction of systems for laboratory animals' anesthesia, electrophysiological and hematological analysis, and internal organ perfusion. Pathomorphological changes in tissues will be studied with modern histological and immunohistochemical methods using light, and fluorescent microscopy instruments.

To clarify the possible mechanisms of radiation-induced CNS damage and cognitive deficit, it is planned to investigate the glial cells' vital role. Demyelination is considered as one of the most probable causes of ionizing radiation-induced cognitive deficit due to the radiation-induced death of oligodendrocytes. Another probable cause is a chronic neuroinflammation caused by activated microglia. Both mechanisms were recently shown to

play a role in different neurodegenerative diseases like multiple sclerosis, Huntington's, Parkinson's, and Alzheimer's. However, no progress has been made in understanding the basis of radiation-induced cognitive disorders.

- *Molecular radiobiological aspects of radiation therapy*

Earlier LRB's research showed that, in the presence of the DNA synthesis inhibitors 1-β-D-arabinofuranosyl cytosine (Ara-C) and hydroxyurea (HU), after γ-ray and accelerated proton exposure the DNA DSB yield significantly increases during post-irradiation incubation of cells due to the transformation of nonlethal DNA damage to enzymatic DSBs. As Ara-C and HU are officinal drugs used for the treatment of acute and chronic leukemia, and, as part of combined or complex therapy, HU is used for the treatment of different tumors (head and neck tumors, skin melanoma, and the colon, rectum, uterine neck, kidney, and prostate cancer), it seems essential to conduct further research on the influence of these agents on the biological effectiveness of ionizing radiations of different quality on different tumor cell cultures and tumors transplanted into mice.

- *Mathematical modeling of radiation-induced effects*

Future work will be focused on the development of a hierarchy of models that would allow systematizing experimental data and studying the pathways of how radiation-induced pathologies develop at different organization levels (from molecules to cell populations) and time scales (acute and long-term radiation effects). methods for theoretical research in this field. It will have to involve a wide range of computational techniques from different knowledge areas (modeling of transport of charged particles through matter, molecular dynamics, biophysics of polymers, genetic regulatory networks, dynamics models of cell populations, processing and transmission of information in neural networks) and computer resources, including the supercomputer of the JINR.

As part of research within the framework of the Theme, typical radiation therapy scenarios will be modeled for different types of radiation: γ-rays and accelerated protons and carbon ions. Examined will be the physical mechanisms that are already used to increase the biological effectiveness of charged particle beams (using nanoparticles as a target increasing the effect) and the most promising biological mechanisms (using DNA synthesis inhibitors).

It is also planned to assess charged particles' influence on the functioning of neural networks of the brain's critical parts. Scenarios will be examined of both acute local exposure (to evaluate radiation therapy safety) and total chronic exposure (to advance in the solution

of the problem of space radiation action on cells during interplanetary flights). It will allow estimating the failure probabilities of different types of memory and learning, which is essential for the theoretical evaluation of the radiation risks.

- *Improvement of accelerator-based radiobiological experiment procedures*

It is planned, first of all, to provide scientific and technological support to radiobiological experiments at charged particle accelerators. Within the framework of the NICA project, it is planned to continue the LRB's participation in the creation of the SODIB station at the Nuclotron, JINR's VBLHEP, for radiobiological research at heavy ion beams with energies of 250–1000 MeV/nucleon. Further upgrade will be continued of the LRB's Genom automated irradiation facility at the U-400M cyclotron, JINR's FLNR.

Radiation safety research will be focused on the LRB's participation in designing JINR's new nuclear physics facilities — first of all, the NICA accelerator complex. Concerning research on radiation fields at JINR's nuclear physics facilities and in their environment, measurements will be continued of neutron spectra at the facilities and in places with the most complex radiation conditions.

In space radiobiology, Monte Carlo calculations of radiation transport in matter will be performed using the software toolkits GEANT4, FLUKA, and PHITS in order to make a realistic evaluation of the cosmonauts' effective doses depending on flight duration, solar activity, and radiation protection of the habitable module.

As part of the joint research program with the Institute of Space Research of the Russian Academy of Sciences and JINR's FLNP, the functioning of the DAN experimental stand will be provided, and the LRB's participation will be continued in the design, fabrication, testing, and calibration of nuclear planetary science instruments.

Work stages

1. Radiobiological research at charged particle beams

Leader: E.A. Krasavin

The LRB's key performers:

Bazlova T.N., Bezhanyan T.Zh., Bogdanova Yu.V., Boreyko A.V., Budennaya N.N., Chausov V.N., Chernyak O.O., Fadeeva T.A., Filatova A.S., Gureu D.-N., Ignat E.M., Ilyina E.V., Isakova M.D., Ivanov A.A., Khramko T.S., Kokoreva A.N., Kolesnikova I.A., Koltivaya N.A., Komarov D.A., Komova O.V., Korogodina V.L., Koshlan I.V., Koshlan N.A., Kovalenko M.A., Kozhina R.A., Kruglyakova E.A., Krupnova M.E., Kutsalo P.V., Kuzmina E.A., Lalkovičova M., Lkhasuren, Lyakhova K.N., Melnikova L.A., Nasonova E.A., Nurkasova A., Ostrovsky M.A., Pavlova A.S., Petrova D.V., Pronskikh E.V., Severyukhin Yu.S., Shamina D.D., Shvaneva N.V., Smirnova E.V., Tiunchik S.I., Utina D.M., Vasilyev L.A., Vinogradova Yu.V., Zadneprianetc M.G., Zhuchkina N.I.

2. Radiation research

Leader: G.N. Timoshenko

The LRB's key performers:

Aleinikov V.E., Beskrovnaya L.G., Gordeev I.S., Komochkov M.M., Krylov V.A., Lesovaya E.N., Pavlik E.E.

3. Mathematical modeling of radiation-induced effects

Leader: A.N. Bugay

The LRB's key performers:

Aksenova S.V., Batova A.S., Chizhov A.V., Dushanov E.B., Enyagina I.M., Glebov A.A., Kolesnikova E.A., Lkhagva B., Munkhbaatar B., Panina M.S., Parkhomenko A.Yu., Tudevdordzh T., Vasilyeva M.A.

4. Training specialists in radiation safety and radiobiology

Leaders: A.N. Bugay, E.A. Krasavin (LRB, JINR); S.Z. Pakuliak (the University Center, JINR)

The LRB's key performers:

Beskrovnaya L.G., Boreyko A.V., Budennaya N.N., Chizhov A.V., Dushanov E.B., Koshlan I.V., Lesovaya E.N., Timoshenko G.N.

Results expected upon completion of the theme

1. Research on the mechanisms of the development of DNA molecular damage and its repair in cultures of human and mammalian normal and tumor cells and in histological sections of tissues of different parts of animals' central nervous system after exposure to radiations of different LET.
2. Research on the induction and molecular nature of different types of gene and structural mutations in mammalian and lower eukaryote cells depending on the radiation dose and LET, repair status, oxidative stress development, and genetic stability mechanisms.
3. Research on the formation of complex chromosomal aberrations in normal and tumor cells of humans and laboratory animals. Evaluation of long-term consequences of exposure to radiations of different LET.
4. Research on behavioral reaction disorders and pathomorphological changes in different structures of the brain, spinal cord, and critical organs and systems of irradiated laboratory animals. Conducting a search for new radioprotective drugs.
5. Research on radiation-induced effects in microglia, oligodendrocytes and their precursors, and in the myelin sheath after exposure to densely ionizing radiation.
6. Research on the mechanisms of the action of Ara-C and other radiosensitizers for the irradiation of different normal and tumor cell cultures and mice with transplanted tumors.
7. Development of an hierarchy of mathematical models of radiation-induced biological effects that would describe the development of radiation-induced pathologies at different organization levels — from molecules to cell populations — and at different times — the acute and long-term consequences.
8. Improvement of accelerator-based radiobiological experiment procedures. Calculation of shieldings for new nuclear physics facilities; evaluation of the radiation conditions and development of radiation safety systems for them. Participation in the creation and tests of nuclear planetary science instruments.

Participating countries, institutes and organizations

Country or organization	City	Institute or Laboratory	Participants
Armenia	Yerevan	YSU	Harutyunyan R.M., Harutyunyan S.G.
Belarus	Minsk	Institute of Physiology NASB IBCE NASB SPMRC NASB	Kulchitsky V.A. Antonevich N.G. Khasanov O.Kh. Gusakov V.E.
Bulgaria	Sofia	IE BAS NCRRP Inst. Microbiology BAS	Avramov LA Khadzhidekova V. Danova S. Tsakov I.
Cuba	Havana	CENTIS	Gonzalez I.
Czech Republic	Brno Řež	IBR ASCR NRI	Kozubek S., Falk M. Stefanik M.
Germany	Darmstadt	GSI	Durante M.
Italy	Naples Udine	INFN UNIUD	Blaha P. Ambesi F.
Mongolia	Ulaanbaatar	NUM	Lhagva O.
Poland	Krakow Szczecin	INP PAS SU	Swakon J., Waligorski M., Olko P., Miszczyk J., Grzanka L. Czerski K., Kowalska A.
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		UMF	Verga N.
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	Iași	IBR	Vochita G., Mihai C.-T.
Russia	Moscow	IBMP RAS	Orlov O.I., Shtemberg A.S., Il'in E.A.
		SF IPh	Kudrin V.S.
		MSU	Latanov A.V., Poletayeva I.I.
		SINP MSU	Panasyuk M.I.
		IKI RAS	Mitrofanov I.G.
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	Obninsk	A.F. Tsyb Medical Radiological Research Centre	Zamulayeva I.A., Khvostunov I.K. Evstratova E.S.
	Pushchino	ITEB RAS	Gaziyev A.I.
	Sochi	SRI MP	Klots I.N.

Serbia	Belgrade	INS “VINČA” Institute for Oncology and Radiology	Adžić M. Zdravković S. Nešković N. Čevizović D. Stanojković T.
Slovakia	Bratislava	CU	Dubnickova M.
South Africa	Cape Town	iThemba Labs	Vandevoorde C.
Vietnam	Hanoi	INPC VAST ITRRE VINATOM	Wu Thi Ha Trinh Thi Thu Huong Le Thi Mai Huong

Time frame of the Theme: 2021–2023.

Expenditures for the Theme according to the Seven-Year Plan for the Development of JINR

	Expenditure items	**Full cost, thousand USD**	**1st year**	**2nd year**	**3rd year**
	Direct expenses				
1.	Materials	240	70	70	100
2.	Equipment	158.2	84.2	43.2	30.8
3.	Payments for agreement-based research	-	-	-	-
4.	Travel allowance	150	50	50	50
	Total direct expenses	548.2	204.2	163.2	180.8

Other financing sources: none

AGREED:

JINR Chief Scientific Secretary
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Theme Leaders

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