DIVISION OF RADIATION AND RADIOBIOLOGICAL RESEARCH

The scientific programme of DRRR is determined by a first priority theme of the Topical Plan for Scientific Research of JINR in two main lines. The radiation field is traditionally connected with radiation shielding design, dosimetry, spectrometry and radiation monitoring. The investigations of peculiarities and mechanisms of mutation in pro- and eukaryotic cells induced by different types of radiation constitute the basis of the radiobiology field at the present time. Besides, the «Mitra» project on the development of new radiopharmaceuticals for target therapy of human melanoma is being successfully realized in the framework of the theme.

RADIATION RESEARCH

The important task of radiation protection at new nuclear installation designing is to minimize the radiation influence of an installation on population and environment. There is no generally accepted method of calculation at large distances of the population exposure resulting from accelerator operations. The most reliable and detailed prediction can be obtained by the statistical modeling method. The other difficult problem is to estimate the activity of the radionuclides escaping to air from an accelerator. The techniques for prognostication of neutron and gamma doses at large distances due to «skyshine» effect and air activity resulting from accelerator operation were developed by using Monte-Carlo codes. The results were applied to the cyclotron centre project in Bratislava (Slovakia).

The data of the experimental investigation of the differential characteristics of neutrons' yield from the thick lead target simulating the core of the SAD assembly were processed and compared with the calculations by the MCNP4B + LAHET and MCNPX codes. The verification of the neutron transport codes were done for the calculation of: 1) the neutron spectra under several angles; 2) the angular and the spatial distributions of the hadron's yield at different energy thresholds. A good agreement between the calculated and experimental data in a wide energy range was obtained.

The calculations of neutron detection efficiency of the NE 213 scintillator were carried out with the modernized code. The precise calculation of the efficiencies of the gamma spectrometers at various detector-source geometry was also carried out. The responses of solid track detectors on the basis of CR-39 and PADC (with fission radiators and without them) to the ^{12}C ions with energies of 0.5 and 1.0 GeV/amu and protons with energies of 0.5 and 1.0 GeV were investigated [1].

The physics support of the biological experiment on the blood lymphocytes and mammalian cells in culture irradiation by the ^{12}C ions with an energy of 0.5 GeV/amu was done at the Nuclotron. For the biological samples irradiation with the particle beams the near uniform radiation field in the area of the samples is formed and the absorbed doses are measured with the calibrated ionizing chamber.

Area and occupational personnel radiation monitoring in the field of the JINR nuclear installation was continued.

The main goal of radiobiological research was connected with investigation of the mutagenic effect of heavy charged particles on bacteria, yeast, mammalian and human cells. The study of regularities and mechanisms of point ($fepA^-$, $tonB^-$) and deletion ($tonB^- trp^-$) mutation induction in bacteria *E. coli* by radiation with a broad region of linear energy transfer (LET) was accomplished. It was shown that the frequency of point mutations as a function of the γ-ray and heavy ion dose (helium and carbon ions with LET 20–200 keV/μm) is described by the linear-quadratic curves. The induction of deletion mutations by helium and carbon ions (LET = 20–200 keV/μm) is described by the linear function. The helium ions with LET = 50 keV/μm are more effective in induction of deletion mutations than the carbon ions (Fig. 1).

The induction of mutagenic SOS repair in *E. coli* cells after irradiation by deuterons, helium and carbon ions was studied by using the SOS lux test. A genetically controlled luminescent bacterial reporter assay was developed for detection of cell SOS response. It was shown that the relationship of SOS induction potency (SOSIP) to LET has a local maximum in a region of 50–60 keV/μm (Fig. 2). The results that were obtained with bacterial cells suggest the important role of cluster DNA damages in the formation of point mutations. On the other hand, the formation of deletion mutations is connected with induction of direct and enzymatic double strands breaks of DNA.

The important data concerning regulation of cell cycle of yeast *Saccahromyces cerevisiae* and sensitivity to DNA damage agents have been obtained in recent years. According to these data, for instance, the RAD9, RAD17, and RAD24 genes are believed to act at the initial steps of damage recognition. The RFC-Rad24 protein complex plays probably a role in loading the Rad17-Mec3-Ddc1-complexes or repair proteins on damaged DNA. The RAD53 protein kinase is involved in a signal transduction cascade, and the other kinase, CDC28, acts at the final step of cell cycle arrest regulation. Arrest is required for repair of damages. The branched system of regulation of cell cycle arrest due to DNA damage needs studies.

It is known that defects in the mechanisms that regulate cell cycle arrest due to DNA damage are believed to have some definite consequences for cell hereditary apparatus, *viz* to cause genetic instability and increased cell sensitivity to DNA-tropic agents. Indeed, enhanced sensitivity to damaging agents has been found in various checkpoint mutants affected in checkpoints. Our and some existing literature data however suggests that *post hoc* may not be completely *propter hoc* here.

Single and double strains were constructed to analyze interactions between the RAD9, RAD17, RAD24, RAD53, and CDC28 genes, and cell sensitivity to γ radiation was determined. The RAD9, RAD17, and RAD24 genes act in one and the same pathway of radiosensitivity, while the RAD9 and RAD24 genes have been classified as belonging to different groups dealing with DNA damage induced by UV light or MMS.

Protein kinases encoded by the RAD53 and CDC28 genes are epistatic to the RAD9 gene but are most probably involved in different pathways of radiation sensitivity

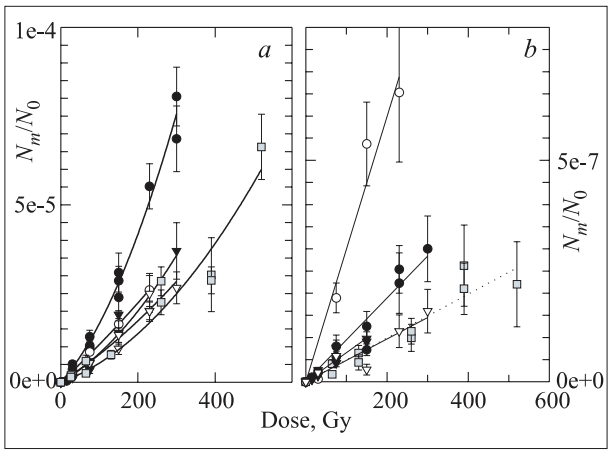

Fig. 1. Frequencies of tonB (*a*) and $tonB^- trp^-$ deletion (*b*) mutations in *E. coli* cells induced by ionizing radiation of different LET. ○ — helium ions (20 keV/μm); ● — helium ions (50 keV/μm); ▼ — helium ions (78 keV/μm); ▽ — carbon ions (200 keV/μm); ■ — γ irradiation (^{137}Cs)

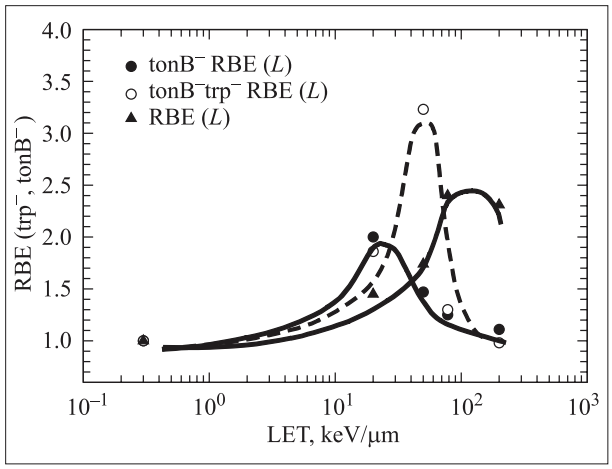

Fig. 2. Dependence of RBE induction of the $tonB^-$ and $tonB^- trp^-$ mutations on LET of radiation in *E. coli* X7026 cells

control, while CDC28 and RAD53 belong to the same branch of the regulation of cell cycle arrest.

Studies continued on induction of mutations of different nature on ionizing radiation in particular point mutations, by using a convenient tester system for 6 possible base-pair substitution diagnostic. Base-pair substitution — transversion AT–TA induced by helium and carbon ions with LET values of 80 and 200 keV/μm was characterized using the diploid strain YMH53. The mutation induction efficiency of He^{2+} (80 keV/μm) was less than the efficiency of He^{2+} (20 keV/μm). The frequency of AT–TA transversion induced by C^{6+} ions was the lowest.

Frame-shift mutations were tested in the strain RKY2672. This strain has insertions in the LYS2 and HOM3 genes, which revert to wild type as a result of only frame-shift mutations. Earlier mutation rate induced by ionizing radiation was measured. Now spontaneous reversion rate is monitored. The rate of spontaneous LYS2 reversion was 10^{-8} and HOM3 reversion — $2.5 \cdot 10^{-8}$. The results are consistent with literature data.

An extended time-course study of expression of chromosomal aberrations induced by radiation of wide LET range was finished at GSI (Darmstadt, Germany), in collaboration with GSI Biophysics Group. In these experiments the time-dependent expression of particle-induced chromosomal damage has been analyzed in 3 Chinese hamster cell lines: V79, CHO K-1 and xrs5 [2]. The cells synchronized by either centrifugal elutriation or mitotic shake-off were irradiated in G_1 phase of the cell cycle by X rays and accelerated heavy ions (Ne, Ar, Kr, Au) obtained at the UNILAC and SIS facilities (GSI, Darmstadt) with LET ranging from 360 to 3980 keV/μm. The chromosome damage has been measured at time span ranging from 10 to 34 h after exposure covering the time interval of almost 3 cell generations, so that almost all dividing cells, including very delayed, were collected. The cells were analyzed at 5–12 subsequent sampling times in 2–4 h intervals preceded by 2 h colcemid treatment. To distinguish between the metaphases of various postirradiation generations the Fluorescence-plus-Giemsa (FPG) technique was applied, and the chromosomal damage was analyzed separately in the first- and second-generation metaphases [3].

The irradiation was shown to retard the cell cycle progression and delay the entry of cells into mitosis, while the expression of chromosomal damage was markedly affected by radiation-induced cell cycle delay in dose- and LET-dependent manner. The number of aberrant cells and aberrations have been found to enhance drastically with sampling time after particle exposure, and this effect depended on LET: the most pronounced increase was observed for Ar and Kr exposure (LET: 1280 and 3980 keV/μm), reaching 100 % of aberrant cells and 10–15 aberrations per cell at later sampling times. The aberration frequency increased in *X*-ray- and Ne-irradiated samples by a factor of 2–3, while after Ar (4.6 MeV/amu, LET 1840 keV/μm) by a factor of 20.

The observed differences in the cell cycle progression and in time course of aberrant cells after exposure of G_1 cells to densely and sparsely ionizing radiation are obviously related to different spatial energy deposition of both radiation types. The applied dose of X rays is homogeneously distributed over the cells. Thus, only small fluctuations in the amount of damage within target cell are induced and synchrony of population is at least partially maintained. In contrast, the particle exposure results in high inhomogeneity of energy deposition both in terms of dose distribution inside the track and in the number of particle traversals per cell nucleus. One of the biological consequences of this non-uniformity of energy deposition by particle exposure is the affected time course of the appearance of chromosomal damage: cells with a low number of particle traversals and correspondingly low chromosomal damage enter mitosis earlier than the cells with a high number of hits and severe chromosomal damage. The distribution of particle-induced aberrations among the cells was fitted by Neyman type A distribution that takes into account the stochastic distribution of particle traversals over the cells as well as the stochastic distribution of aberrations per single traversal. The fit parameters clearly reflected the damage-dependent cell cycle delay: the number of particle traversals per cell appears to increase with sampling time, i. e. highly hit cells are delayed for a longer time and carry correspondingly more aberrations than the cells with low number of particle traversal, less damaged which reach mitosis earlier.

Thus, the RBE (relative biological efficiency) values of high-LET particles obtained for single sampling time were shown to be strongly dependent on time: for example, after Ar ion exposure they varied from 0 at 14 h (standard fixation time) to 4.0 at 22 h postirradiation and could thus be misleading. It became clear that for the correct determination of significant RBE values multiple fixation regimes should be used. To account for the time-dependent expression of chromosomal damage, a novel mathematical approach has been developed [4–7]. This approach allowed one to quantify the amount of damage within the whole cell population. The method was shown to be more adequate for the comparison of experimental data obtained for different radiation qualities which caused different cell cycle perturbations compared with the conventional methods based on single sampling time data. It yielded for Ne (LET 460 keV/μm), Ar (1226 and 1840 keV/μm) and Kr (3980 keV/μm) ions values of 3.2,

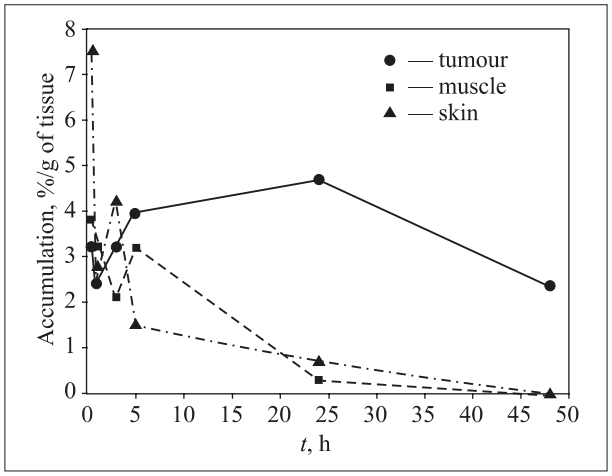

Fig. 3. Accumulation of ^{131}I-MTB in tissues of tumour-bearing animals

1.9, 1.4, 1.3, respectively, which were much higher than all previously published RBE values obtained routinely at single fixation regime in this region of very high LET [6].

The mathematical method was also applied to reconstruct the growth curves and to evaluate the amount of aberrant cells, which reached mitosis during the course of experiment as well as the number of lost cells due to the incomplete sampling or interphase death. It was demonstrated that in case of Chinese hamster cells used here the vast majority of exposed cells were able to reach their first postirradiated mitosis despite pronounced cell cycle delay.

Finally, LET-dependent alterations in aberration spectrum were demonstrated in line with the general view that the quality of lesions underlying chromosomal aberrations changed with LET. First, it was shown that after high LET radiation the chromosomal breaks were preferentially formed, while after sparsely ionizing radiation the aberration spectrum was dominated by exchange-type aberrations like dicentrics: the fraction of chromosomal breaks increased from 40 % after X-ray exposure to 55–65 % for particles with LET in the range 1000–4000 keV/μm. A further characteristic feature of densely ionizing radiation was the occurrence of a high number of chromatid-type aberrations despite cell irradiation in G_1 phase of the cell cycle. While after X irradiation the chromatid-type aberrations accounted for about 10–15 % of aberration yield, this amount gradually increased with LET being 30 % for Ne (LET 460 keV/μm), 35–40 % for Ar (1226 and 1840 keV/μm) and 45 % for Kr (3980 keV/μm) ion exposure. This is a consequence of higher complexity and lower reparability of DNA lesions produced by high LET radiation. As an extension of these studies, the time-course of chromosomal damage induced by radiation of different quality in normal healthy human fibroblast cell lines as well as in human peripheral blood lymphocytes is currently investigated [7].

The investigation of low-dose radiation effects was continued. The induction of cytogenetic damage after irradiation of Chinese hamster cells and human melanoma cells within a dose range 1–200 cGy was studied [8–10]. The anaphase and metaphase analyses of chromosome damage and micronuclei test were applied in asynchronic and synchronic populations. The hypersensitivity (HRS) at doses below 20 cGy and the increased radioresistence (IR) at higher doses were shown for all cytogenetic criteria in both cell lines. The phenomenon of HRS/IR was reproduced in synchronic as well as in asynchronic population of Chinese hamster cells. This reflects that HRS was caused by high radiosensitivity of all cells and cannot be explained by changes of radiosensitivity of cells in different phases of the cell cycle. So it was supposed that the increasing radioresistence is determined by the inclusion of the inducible repair processes in all cells. This conclusion consents with the fact that there was no evidence of HRS on dose-effect curves and that some parts of pre-existent damage were repaired after preliminary irradiation with low doses (1–20 cGy) which induced repair processes. It can be concluded that the same inducible repair processes underlie either the HRS/IR phenomenon or adaptive response [11].

Comparison of radiobiological effective depths with 150-MeV unmodulated proton beams (FLNP) was conducted. Cell survival curves were generated with the *in vitro* colony-forming assay. With ^{60}Co gamma rays as the reference irradiation, the relative biological effectiveness values for a survival fraction level of 0.1 at Bragg peak and plateau are 1.17 and 1.03, respectively. So, to maintain uniformity of radiobiological effectiveness for a target volume, careful attention should be paid to the influence of the depth of beam and irradiation dose.

As an extension of the previous studies on the genotoxic effects of low doses of radiation and formation of stable and unstable chromosomal aberrations, an experiment was performed on mammalian cultured cells and human lymphocytes exposed by ^{12}C ions (480 MeV/amu) obtained at the Nuclotron (LHE).

Studies on targeted therapy of human pigmented melanoma with radiolabeled methylene blue (MTB) were continued. Experiments were carried out with MTB labeled with ^{131}I or ^{211}At *in vitro* and *in vivo*. Accumulation of ^{131}I-MTB in human pigmented melanoma cells *in vitro* is about 4–5 times higher than that in non-pigmented cells (Fig. 3). The maximum of accumulation occurs in 2 h after injection [12]. The obtained data stay in accordance with the previous results on ^{211}At-MTB accumulation *in vitro*.

Experiments *in vivo* show rapid excretion of ^{131}I-MTB from all organs of tumour-bearing mice during first 24 h. Accumulation of the compound in tumour has maximum (5 %/g of injected activity) in 24 h and remains at a high level during at least first 2 days after injection. Tumour to normal tissues ratios for ^{131}I-MTB accumulation were 36:1 for blood, 47:1 for muscles, 7.8:1 for skin at 24 h after i. v. injection [13, 14]. Preliminary data on ^{211}At-MTB biodistribution shows high accumulation of the compound in melanoma (6 %/g of injected activity) at 5 h after injection and its slow excretion from the other organs.

The analysis and comparison of different models for describing radiobiological effects at low-dose exposure have been done [15]. All the models are presented by linear nonthreshold term of damage yields, which is resulting for the linear nonthreshold model. This fact reflects the regularity that primary reason for all stochastic radiobiological effects (perhaps DNA breaks) is linearly and nonthreshold-dependent on dose. However, the linear term of effect is necessary but insufficient for adequacy of the model, in general case, to the observation result. The linear-quadratic model can be adequate to any data on a cell level to a dose of more than 1 Gy, however at less doses the adequacy often breaks. The inducible repair model is adequate to the data on a cell level. The confidence level of the model of two cell populations is selectively adequate to the data on a cell level. The confidence level of the model of two protection reactions on totality of data is the highest one of the compared models and suitable for all the levels of an organism. On the basis of this model the estimation of radiation risk for population of the most contaminated regions of Belarus has been done [16].

CONFERENCES

The II International Symposium under the auspices of UNESCO «Problems of Biochemistry, Radiation and Space Biology» dedicated to the memory of Academician N. Sissakian and the II N. Sissakian Readings were held at the A. Bakh Institute of Biochemistry (Moscow) and at JINR on 29 May – 1 June 2001. About 120 participants from Russia and other European countries attended the Symposium. The Symposium Scientific Programme included 16 plenary reports and more than 80 reports were distributed among three special sessions on: biochemistry; space biology and medicine; general and space radiobiology.

A competition of the reports among the young scientists was organized in the framework of the Symposium. The winners were awarded diplomas and premiums.

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