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EXPERIENCE OF EXPERIMENTAL RESEARCH ON RADIATION ONCOLOGY AT R.E. KAVETSKY INSTITUTE OF EXPERIMENTAL PATHOLOGY, ONCOLOGY AND RADIOBIOLOGY, NAS OF UKRAINE

This article briefly summarizes our long-term experience of research in the field of experimental and clinical radiation oncology unified by the key word "radiosensitivity". Consistently presented and interpreted here are the main results on biodosimetry of irradiation depending on doses and quality of ionizing radiation and determination of individual radiosensitivity of cancer patients. We justified the use of radiomitigators to reduce the frequency and severity of post-radiation complications in cancer patients, and for radiation protection of the general population. The radioprotective effect of the antioxidant inosine in the somatic cells of cancer patients in the range of low radiation doses was demonstrated. We established that in persons who are hypersensitive to irradiation, the reparative potential is reduced by about 60% compared to ones with normal indices of individual radiosensitivity. Cytogenetic predictors of radiosensitivity of healthy cells adjacent to the irradiated tumor have been determined. Unfortunately, they have not yet become a point of application for individual planning of irradiation courses and assessment of severity of post-radiation complications. The intensive development of selective radioprotectors that would selectively protect healthy tissues in the course of radiation therapy, reducing their radiosensitivity by activating reparation processes, is considered a priority direction of modern radiation oncology.

Keywords: ionizing radiation, therapeutic exposure, individual radiosensitivity, radiomitigators, cancer patients, carcinogenic effects.

Radiation oncology is one of the most science-intensive medical disciplines based on the latest achievements of radiobiology, chemistry, radiation physics and engineering, radiation hygiene,

mathematics, etc., which develops priority methods of cancer treatment. One of the most important strategic directions of modern radiation oncology is the improvement of therapeutic

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irradiation methods to achieve complete tumor destruction while maintaining an acceptable level of the damage to normal cells. The progress in the technical means for irradiation has occurred due to the introduction of new-generation accelerators into the oncological practice, which allows focusing ionizing radiation (IR) beams with a millimeter accuracy, to irradiate tumors in the mode of spatial and temporal modulation, to achieve the conformal irradiation strategy, etc. In Ukraine, a regional imbalance in properly equipped radiation oncology centers exists, and post-Soviet devices are still used [1]. Therefore, an important task of modern radiation oncology in Ukraine is its technical re-equipment, including the acquisition of new-generation gamma-therapeutic devices with the option of 3D-conformal radiation therapy.

The radiobiological basis for the use of radiation therapy in oncology is the therapeutic interval — the difference in the degree of damage and recovery of tumor and normal tissues at the equal levels of the absorbed IR doses. Hence, the main requirement for therapeutic irradiation of cancer patients is to concentrate the maximum IR dose in the pathological focus with the minimal damage to normal tissues surrounding the tumor. However, radiosensitive tumors are destroyed with minimal damage to the surrounding healthy tissues (tumor bed), while in radioresistant tumors, radiation doses cause significant damage to healthy tissues along with the tumor. Therefore, the tasks of radiation oncology are not only to overcome radioresistance of tumor cells but also to minimize radiation complications arising from therapeutic irradiation [2].

Researchers note that the possibilities to improve the results of radiation therapy of cancer only by improving its physical and technical equipment are largely exhausted [3]. We believe that this is partly explained by the shift of research interests in the post-Chornobyl period toward the study of the peculiarities of low-dose IR effects. As a consequence, the attention to the

biological effects of the high doses used for cancer treatment has been weakened.

The main radiologic research of the Institute in the recent decades was focused on the following:

- assessment of IR biodosimetry in a wide range of doses depending on the radiation quality;
- study of individual radiosensitivity (IRS) of cancer patients;
- use of radiomitigators to reduce the incidence and severity of the post-radiation complications in cancer patients, as well as the radiation protection of the general population in case of nuclear threat.

It is convincingly shown that the introduction of new types of IR into practice necessarily includes the study of the peculiarities of the quality of IR and the nature of dose dependence and their influence on the formation of radiation reactions. Among quantitative radiobiological regularities, the most pronounced relationship exists between the radiation dose and the magnitude of the effect, i.e. the dose-effect relationship.

There are several reasons for the occurrence of post-radiation complications due to therapeutic irradiation of cancer patients, the main ones being the following.

Firstly, malignant tumors form microscopic infiltrates in healthy tissues, which must be included in the irradiated volume. Secondly, the cells of these tissues at the input and output of the IR beam are also subjected to radiation changes that affect their functional status, including radiosensitivity. As a consequence of therapeutic irradiation, the active toxic products of the radiation decay of tumor cells are "washed out" into the bloodstream, which causes the structural and metabolic disorders in the patient's organism.

A number of studies attribute the reasons for the development of radiation complications in healthy tissues to the errors in planning the course of radiation therapy, when in some cases

high single and total doses are administered (the "double anxiety" site), which exceed the tolerance of these tissues to IR. At the same time, the peculiarities of absorbed dose distribution in the tissues surrounding the tumor are not always correctly taken into account as well as the IRS of patients. The molecular, chromosomal, biochemical, and other abnormalities in healthy cells of primary cancer patients give grounds to consider them only conditionally normal [4]. The risk of the distant radiation reactions can be high, taking into account the stochastic, including carcinogenic character of their formation [5]. Therefore, irradiation of cells of healthy organs and tissues during radiation therapy of primary cancer in 15% of cases may cause secondary cancer of radiation etiology. Predisposing factors for the development of increased radiation response can be high IRS, previous inflammatory processes, diabetes mellitus, thyrotoxicosis, biological features of the tumor, and a number of other factors [6].

The distant post-radiation complications in critical vital organs reduce the effectiveness of radiation therapy, as well as the quality of life of patients. One of the main approaches to optimizing the existing methods of therapeutic irradiation of patients is to determine and take into account their IRS, which predisposes radiation adverse reactions and determines their severity [7]. Therefore, our idea is not only to select a group of patients with high IRS, but also to personalize radiation treatment and to manage radiosensitivity using effective and nontoxic radioprotectors.

G2-radiation sensitivity assay in our modification on the basis of classical provisions of radiation cytogenetics is the most informative method to assess the potential IRS of cancer patients. As a result of our *in vitro* studies of radiosensitivity of peripheral blood lymphocytes (PBL) during the mitotic cycle and the nature of the dose-effect relationship, we have developed an algorithm for the assessment of IRS, a detailed de-

scription of which is presented in [6]. In contrast to the methodological approach in other studies, we suggest performing chromosome analysis during the period when the predominant part of cultured PBLs divides for the first time. In subsequent mitoses, the frequency of chromosome aberrations decreases, which may result from the elimination of aberrant cells, radiation delay of mitosis, reproductive cell death, or changes in the spatial arrangement of chromosomal aberrations. We have shown for the first time that the frequency of radiation-induced aberrations in the low dose range (0.1—0.5 Gy) for more radiosensitive patients with cervical cancer is 4 times higher than that for radioresistant ones. Also, cytogenetic parameters of more radiosensitive patients reach a plateau (dose-independent area) on the calibration curve located significantly higher than those of less radiosensitive cancer patients.

The response of healthy tissues to therapeutic irradiation showed prominent differences between individuals [8]. We have shown that there is a correlation between the increased frequency of radiation-induced chromosomal aberrations in immunocompetent cells (T-lymphocytes) detected *in vitro* and the predisposition to cancer development [9].

We have developed and suggested a new methodological approach to assess the contribution of repair processes to the formation of IRS, based on the additional effect of hyperthermia in the S-phase of the mitotic cycle. First, our studies confirmed the pronounced radioresistance of human lymphocytes during the period of chromosome replication (S-phase). Second, preliminary studies of the cytogenetic effect of hyperthermia in the S-phase were performed based on the following considerations. Since at this cell cycle phase, the activation of repair processes takes place, the inhibition of them and, accordingly, higher radiation damage should be expected under an additional thermal action. We have established that the frequency of chro-

mosome aberrations induced in the S-phase of cultured PBLs of individuals with normal IRS was 22.6 ± 2.0 per 100 metaphases, while in the group with high IRS 31.0 ± 2.1 per 100 metaphases. Additional post-radiation hyperthermic action in the S-phase caused an increase in these indices at average of 50.5 ± 1.8 per 100 metaphases and 43 ± 1.1 per 100 metaphases, in the normal and high IRS groups respectively (i.e. the coefficients of thermal enhancement of cytogenetic effect were 2.2 and 1.4 respectively). So, in individuals hypersensitive to radiation, the repair processes are insufficiently expressed compared to individuals with the average values of IRS.

Thus, the reparative potential of individuals hypersensitive to radiation is reduced by about 60% compared to the group of individuals with normal IRS values. At the same radiation dose, the frequency of dicentrics and centric rings, markers of radiation exposure, differs. The greatest fluctuations in radiosensitivity are observed in groups of large chromosomes (n. 1, 2) compared to other chromosomes in the genome. The results obtained indicate the key role of repair processes, namely their inhibition, in the formation of increased IRS [10].

In accordance with the provisions of clinical radiobiology [11], the main requirements for predictors of cell radiosensitivity include a clear relationship between the radiation dose and the effects that are observed in a short period of time after irradiation. We determined the cytogenetic predictors of radiosensitivity of non-malignant cells, using as a model peripheral blood lymphocytes in endometrial cancer patients. In vitro test irradiation of the blood samples of cancer patients in the dose range of 0.5–3.0 Gy increased the frequency of chromosome aberrations by 2.1–5.6 times with the dose dependence of the cytogenetic effect approximated by a linear-quadratic equation. Such a strict dose dependence of the total frequency of the induced chromosome aberrations in blood lymphocytes of cancer patients allows us to consider this model as a pre-

dictor of the sensitivity of radiosensitive tissues prior to radiation therapy [9, 12]. In fact, this can be a new strategy for the prevention of radiation complications with radiobiological approaches to the identification of cancer patients with high radiosensitivity of the tissues adjacent to the irradiated tumor. Unfortunately, at present, the data obtained regarding the IRS of the organism of cancer patients and predictors of radiosensitivity of healthy cells surrounding the irradiated tumor have not yet become a point for the individual planning of irradiation courses and the assessment of the severity of post-radiation complications [2, 12].

Scientific and practical interest is aroused by the prospect of using microbeam technologies for local irradiation of microvolumes corresponding to the genetic structure of cells [13]. This allows implementing a new step for the creation of high-precision radiation therapy for cancer patients, dosimetry planning, and in-depth study of the mechanisms of radiation carcinogenesis. Since one of the main targets of IR action is the genetic apparatus of cells, its damage indicates qualitative and quantitative characteristics of irradiation. For radiobiological characterization of X-ray and proton microbeams, first of all, the dose-effect relationship, we used PBL culture, an important advantage of which is that it allows us to conduct experimental studies directly on human cells [14].

Linear and linear-quadratic "dose-effect" models traditionally used in biodosimetry do not give a satisfactory approximation to the true functional dependences in a number of cases [15]. It is known that the repair processes that condition the final radiobiologic process are formed due to the compensatory capabilities of the object. We believe that for estimation of radiation-induced cytogenetic effects, it is advisable to use more dynamic models (e.g. a spline regression model). In this case, the final choice of a mathematical model for biodosimetry is determined by the minimum value of the error [16].

We have obtained data on the approximation of the experimental dose-effect relationship at the chromosomal level of human somatic cells (T-lymphocytes) on the basis of a spline regression model, which improves an accuracy of irradiation biodosimetry. This is achieved by reducing the error in determining the value of the absorbed dose in comparison with the traditional use of linear and linear-quadratic models and allows us to predict the effect of the dose curve's reaching a plateau. The radiobiological characterization of the above-mentioned IR microbeams in a wide dose range at the genetic level of highly radiosensitive cells of the human T-lymphocytes studied by us was the justification for the creation of a new method of using IR microbeams in cancer treatment [17].

The use of IR as a sole treatment is, in some cases, ineffective due to the presence of IR-resistant tumor cells. Radiation therapy with fast neutrons is considered to be indicative for approximately 30% of cancer patients. The therapeutic use of neutrons has several advantages such as less dependence of their action on cell cycle periods, the low level of repair of sublethal damage, etc. We conducted preclinical studies on the dynamics of the relative biological effectiveness (RBE) depending on the dose of 6 MeV fast neutrons during the mitotic cycle by the level of radiation-induced chromosome aberrations in PBL culture. The analysis of the cytogenetic data obtained earlier showed that under the action of fast neutrons, the radiosensitivity of human cells varies qualitatively and quantitatively during the mitotic cycle. This proves the existence of repair processes under the action of neutrons of this energy [18]. When exposed to 6 MeV neutrons, the frequency of chromosome aberrations in PBLs increases linearly with the dose, which is confirmed by calculating the parameters of the linear-quadratic model. When fast neutrons with energy of 22 MeV are used for neutron therapy of deep-lying tumors, the frequency of the aberrations in lymphocytes also increases with the radiation dose.

Our results indicate the radioresistance of cells in the S-phase of cell cycle and the lowest values of neutron RBE. The highest RBE value is reached at low doses and in the G2-phase. Neutrons of a wide energy spectrum are 1.6 times more effective than monoenergetic neutrons of the same energy in inducing chromosomal damage in PBLs. This indicates that a significant contribution to the total radiation dose is made by neutrons of intermediate energies, characterized by high efficiency. Our experimental data on the radiobiological characteristics of fast neutrons have contributed to the practical application of neutron therapy to Ukrainian cancer patients. The clinical results obtained via neutron treatment surpassed those obtained by ^{60}Co therapy, especially in radioresistant forms of tumors [18]. Unfortunately, in Ukraine, the priority of neutron therapy for the treatment of cancer patients has not been maintained. Nevertheless, the specialists in the field of radiation oncology and clinical radiobiology hope that in the foreseeable future Ukraine will restore a scientific and clinical base for intensive research using the latest technologies of neutron therapy for cancer patients.

At present, the strategy of radiation protection of general population dictates the necessity to take into account the experience of previous radiation accidents and incidents during which a significant part of the population was exposed to low doses of radiation. Therefore, such a strategy should be aimed at selecting effective and low-toxic radioprotective agents.

We have proved, both theoretically and experimentally, the radiomodifying effect of a number of substances, such as caffeine, verapamil, thymalin, melanin-glucan complex, and others. The main factor that limits a practical use of antiradiation agents is their side effects (hypodynamia, hemodynamic disorders, headache, etc.). The radiomitigators generally do not cause as many side effects. These drugs are capable of attenuating the damaging effects of IR on criti-

cal highly radiosensitive systems of the human body [19]. A special attention is paid to the antioxidant inosine. It has a multivariate mechanism of action resulting in an increase of general radioresistance and mobilization of protective resources of the organism.

We have established that in vitro exposure of PBL of conditionally healthy individuals to the therapeutic concentration of inosine before gamma-irradiation reduces the frequency of radiation-induced aberrations in the whole IR range studied (0.1—1.0 Gy). The greatest radioprotective effect was registered in the range of low IR doses of 0.1—0.3 Gy and significantly reduced the level of radiation-induced chromosome aberrations to the values of the population

average spontaneous level of genetic damage (biomarkers) in radiosensitive (T-lymphocytes) human cells [20]. The obtained results should be regarded as an experimental justification for the targeted use of radiomitigators in radiation oncology to protect normal tissues during therapeutic irradiation of tumors, as well as in emergency situations of irradiation of population to minimize the occurrence and development of stochastic effects.

The priority direction of modern radiation oncology is an intensive search for and development of radioprotectors that would selectively protect healthy tissues around the tumor, reducing their radiosensitivity by activation of repair processes.

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ДОСВІД ЕКСПЕРИМЕНТАЛЬНИХ ДОСЛІДЖЕНЬ З РАДІАЦІЙНОЇ ОНКОЛОГІЇ В ІНСТИТУТІ ЕКСПЕРИМЕНТАЛЬНОЇ ПАТОЛОГІЇ, ОНКОЛОГІЇ І РАДІОБІОЛОГІЇ ІМ. Р.Є. КАВЕЦЬКОГО НАН УКРАЇНИ

У цій статті коротко відображено наш багаторічний досвід досліджень у галузі експериментальної та клінічної радіаційної онкології. Його етапи об'єднує ключове слово "радіочутливість". Послідовно представлено та інтерпретовано основні результати біодозиметрії опромінення в широкому діапазоні доз і залежно від якості іонізуючого випромінювання та визначення індивідуальної радіочутливості організму онкологічних хворих; обґрунтовано використання радіомітигаторів як в онкології з метою зниження частоти й тяжкості постпроменевих ускладнень, так і для радіаційного захисту населення в разі ядерної загрози. Так, уперше встановлено радіопротекторну дію антиоксиданту інозину в соматичних клітинах онкологічних хворих в інтервалі малих доз опромінення — зниження рівня радіаційно-індукованих аберацій хромосом до значень спонтанного. Вперше встановлено, що в осіб, гіперчутливих до опромінення, репараційний потенціал знижений приблизно на 60% порівняно з групою осіб з нормальними показниками індивідуальної радіочутливості. Визначено цитогенетичні предиктори радіочутливості здорових клітин з оточення опромінюваної пухлини. На жаль, їх ще не використовують у променевій терапії під час індивідуального планування курсу опромінення та оцінки тяжкості постпроменевих ускладнень. Пріоритетним напрямом сучасної радіаційної онкології вважаємо продовження інтенсивного пошуку і розробку радіопротекторів селективної дії, які б вибірково захищали тільки здорові тканини з оточення опромінюваної пухлини, знижуючи їхню радіочутливість шляхом активації процесів репарації.

Ключові слова: іонізуюче випромінювання, терапевтичне опромінення, індивідуальна радіочутливість, радіомітигатори, онкологічні хворі, канцерогенні ефекти.