CHORNBOYL CATASTROPHE: CYTOGENETIC EFFECTS OF LOW DOSE IONIZING RADIATION AND THEIR MODIFICATION

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Among the long-term effects of the Chornobyl disaster the greatest concern of international medical and scientific community is given to the established fact of excess of the spontaneous level of cancer incidence in the exposed population. According to modern concepts, the accumulation of chromosome aberrations, especially in radiosensitive cells, could be potentially oncogenic, and low doses of ionizing radiation could be promoters of the radiation-induced carcinogenesis. The results of our studies have shown that such substances as thymalin, inosine, ascorbic acid, caffeine could modify radiation-induced cytogenetic effects in peripheral blood lymphocytes of healthy individuals and exert protective or sensitizing action dependent on their concentrations, cell radiosensitivity, dose of irradiation, and relative biologic effectiveness of ionizing radiation. This article is part of a Special Issue entitled “The Chornobyl Nuclear Accident: Thirty Years After”.

Key Words: ionizing radiation, low doses, modification, stochastic effects, cancer, chromosomal aberrations.

30 years ago the global accident at the Chornobyl nuclear power plant has caused long-lasting ecological problems over vast areas of Ukraine, Russia and Belarus. According to the official figures, currently there are about 10 mln permanent residents on the territories of these countries with radionuclide contamination; tens of thousands of people work in the 30-km Chornobyl exclusion zone, at nuclear power plant in the conditions of increased radiation hazard [1]. There is an increase of the likelihood of human exposure to ionizing radiation (IR) in low doses, which causes stochastic effects, including carcinogenic ones. At present time, special attention should be given to the transuranic elements (metastable radionuclides) the negative impact of which on the health of population will enlarge in even more remote post-Chornobyl period.

IR belongs to the most powerful immunosuppressants and carcinogens capable to exert neoplastic potential at all stages of tumorigenesis. IR can induce the appearance of new tumors and accelerate the development of already existing neoplasms initially not related to irradiation [2], therefore the Chornobyl disaster is considered to cause enhanced carcinogenesis [3]. The doses lower than 0.03 Gy, which are slightly higher than the levels of background radiation, could cause malignant transformation of cells [4]. One of the main paradigms of radiobiology is the reasoned division of the biological effects of radiation into stochastic or non-stochastic. The latter were officially named as “deterministic” and could be observed if the damage to functioning cells is significant.

Stochastic effects of IR are characterized by a linear non-threshold dependence of the probability of their occurrence on the IR dose. At the same time, only the frequency of analysed events depends on the dose of IR, but not their severity. This means that even the most minimal radiation exposure increases the likelihood of stochastic effects. These are, in fact, the effects of low dose IR [5–7]. Stochastic effects include chromosomal aberrations, point mutations, malignant transformation of cells, and the radiobiological reactions without a dose threshold. If deterministic effects of the Chornobyl disaster (general somatic diseases) are implemented up to the level of decompensation of 25-year post-accident period, stochastic effects would not have any limitation period [8].

Several radiobiological studies have shown that exposure to low doses induces genomic instability, gene mutations, chromosomal aberrations, the formation of reactive oxygen forms, decrease (adaptive response) or increase in sensitivity to subsequent mutagenic effects, repair stimulation, “bystander” effect, the damage of membranes under the influence of free radicals (Petkau effect), and other effects. It should be emphasized that the combined effect of mutagenic and carcinogenic agents of radiation and chemical nature at low doses on the human body is almost inevitable. As a result of this combination, irradiation at low doses can be particularly dangerous because it modifies many reactions of the human organism.

Among the long-term effects of the Chornobyl disaster the greatest concern of the international medical and scientific community is given to the established fact of excess of the spontaneous level of cancer incidence in the exposed population. In recent years, the debate has intensified about the development of stochastic effects — radiation carcinogenesis — in the range of action of low doses. The issue of dose dependence of the radiogenic cancer occurrence is an extremely complex and relevant. A paper [9] provides the details on the radiation-epidemiological data obtained from a representative sample of Chornobyl liquidators, which indicate that “low-dose absorbed IR is a statistically significant cancer risk factor”. This concept has supporters [10–12]. Although the opposite point of view, i.e. the positive effect of IR on the
The problem of radiogenic cancer largely lies in understanding the mechanisms of action of low dose IR on the genome, tissues, organs and organism as a whole. Careful attention to this problem is due to the following issues. Firstly, the effects of low dose IR are the promoters of carcinogenesis. Secondly, the radiation effect on oncogenesis at low dose range may be greater per unit dose as compared to the effects of large doses. This is explained by the fact that in the first case the cell death is less pronounced and, therefore, the reparative processes are not stimulated by the cell death; induced genomic instability alters the sensitivity of the irradiated cells to the action of other damaging factors.

Accounting the aforementioned, it is important to focus the attention of specialists in the field of radiobiology and experimental oncology, not only on the characteristics and mechanisms of formation of medical and biological effects of low dose IR, but also on their modification by the agents of chemical nature. Taking into account the radioecological crisis in the post-Chernobyl period, it is important to focus on the search for the ways to protect people from chronic exposure to low intensity radiation.

We have developed and proposed a new classification of radioprotective drugs with the “modernization of the relevant terminology and differentiation of key definitions of the mechanisms of radioprotective agents” [17]:

- **radioprotectors (chemical protection)** — anti-radiation drugs that exert a protective effect at the physico-chemical and biochemical level, preventing the “oxygen effect” as radiobiological phenomenon of absorption of IR energy during radiolysis of DNA;

- **radiomitigators** — anti-radiation drugs that exert their effect at the system level “by accelerating post-radiation recovery of radiosensitive tissues through the activation of a number of antiinflammatory signaling pathways and increased secretion of hematopoietic growth factors, which are used from the early period after exposure until the development of clinical manifestations of acute radiation injury...” [17]. The highest activity of the drugs of this group appears almost exclusively during radiation injury of the hematopoietic system. Radiomitigators include steroid hormones and their non-steroidal structure analogues; adjuvants of immunological responses (vaccines, endotoxins, polysaccharides, polynucleotides, etc.); cytokines (tumor necrosis factor, growth factors, interferons, etc.); immunoregulatory peptides (thymalin, thymogen, taktivin, timoptin, etc.). The mechanism of radioprotective effect of these compounds is related to their ability to accelerate the post-radiation regeneration of the cells of hematopoietic system [18–20];

- **radiomodulators (biological protection)** — pharmaceutical substances and food supplements, which increase the body’s resistance to adverse environmental factors, including IR [21]. This is a large group of natural compounds with antioxidant, antimitogenic, and antiinflammatory activities [22]. The mechanism of their action is characterized by an increase of general (nonspecific) resistance of the organism with a decrease of cancer risk [23].

Recently, the research of radiobiologists has being focused on radioprotective action of cytokines that regulate the growth, differentiation, functional activity and radioresistance of cells [24]. The protective effect of cytokines is determined by their hemodynamic and immune stimulatory activity as well as their ability to increase the endogenous background of radioresistance, enhancing antitumor response [25].

The organic selenium compounds (for example, selenotetracysteine) are recognized as prospective means for prevention of radiation injuries, due to the low toxicity [26]. The antioxidant mechanism of their action is a common characteristic to the formation of all biological effects of selenium and is carried out by selenium containing proteins [27, 28]. Currently, the purine compounds (xanthosine, caffeine, inosine) are considered as prospective preventive agents to reduce the radiation-related risks [29]. It is shown that the efficiency of repair processes by purine compounds is associated with the activation of the poly(ADP-ribose)polymerase — one of the key enzymes in DNA repair [30].

The following are the results of our research carried out at the chromosomal level on human peripheral blood lymphocytes (PBL) — biological indicators of irradiation. Our studies were aimed on clarifying the nature of the modifying (enhancement/attenuation) effects of low dose IR in order to improve the radioresistance of the human genome.

**Thymalin.** A study of the effect of radiomitigator thymalin [17] on the radiosensitivity of PBL chromosomes of healthy people is required for overcoming the negative impact of the Chernobyl accident. Thymalin is a complex mixture of peptides isolated from mammalian thymus. It belongs to the drugs that increase the stability of genome and stimulate the immune and reparative systems [31]. According to the cytogenetic data, 1 h preincubation of T lymphocytes from peripheral blood of apparently healthy individuals with thymalin demonstrated a radioprotective effect of the drug. In particular, thymalin at prophylactic concentration (0.002 mg/ml of blood) decrease the frequency of chromosomal aberrations upon irradiation at a dose of 0.2 Gy from 5.0 ± 1.3 to 2.0 ± 0.9 per 100 cells, and at a dose of 0.5 Gy by 2-fold — from 8.0 ± 1.0 to 4.0 ± 1.0. We supposed that the observed radioprotective effect of thymalin could be related to its stimulating effect on the primary repair of radiation-induced damage. This cytogenetic data allows us to recommend thymalin for the persons at the increased carcinogenic...
risk, especially those exposed to radiation after Chornobyl accident.

**Inosine.** Purine nucleoside inosine is a drug of natural origin used in cardiac practice as a stimulant of metabolic processes, a precursor of the synthesis of adenosine triphosphate and nucleotides that supports energy balance in various tissues [32]. There are established hemostimulating [33], immunotrophic [34], antitumor [35], and antioxidant activities [36] of this drug as well as its ability to stimulate the enzymatic repair of radiation-induced DNA damage [32]. It is assumed that the effect of inosine on repair processes in irradiated cells prevents the formation of intra- and interchromosomal rearrangements. It can be concluded that inosine exerts multifaceted action in a wide range of biological activities, including improving overall radioresistance.

We have found that the level of spontaneous chromosomal aberrations in PBL of healthy individuals was 1.6/100 metaphases (mean population level), and under the influence of inosine administered at a therapeutic concentration (0.01 mg/ml of blood) was 0.6/100 metaphases, i.e. this agent decreased the frequency of spontaneous aberrations by 2.6 times. Also, inosine decreased the level of chromatid aberrations by 1.7 times, and caused the "disappearance" of the chromosomal rearrangement. The introduction of inosine in the therapeutic concentration in the cultures of PBL from healthy individuals before γ-irradiation reduced the incidence of radiation-induced aberrations over the entire dose range (0.1–1.0 Gy). The highest radioprotective effect of inosine was observed when the cell culture was irradiated at low doses of 0.1–0.2–0.3 Gy. The level of radiation-induced chromosomal aberrations decreased from 6.06 ± 0.6; 7.06 ± 1.6; 7.76 ± 1.0 to 1.6 ± 0.1; 2.6 ± 0.4; 2.2 ± 0.6, respectively, thus reaching the values of average population level of spontaneous genetic damage in radiosensitive human T-lymphocytes. Therefore, inosine has an antimutagenic and radioprotective effects during the irradiation of non-malignant radiosensitive cells at low dose range (0.1–0.3–0.5 Gy), reducing the level of radiation-induced damage to the spontaneous genetic values, thereby increasing the resistance of the human genome.

A significant danger is created by the compounds that increase the radiosensitivity of human genome, especially by irradiation at low doses. These include co-mutagens, whose effect on the irradiated cells remains scarcely analyzed. Therefore, we studied in detail the effect of the potential co-mutagen ascorbic acid (AA) on the radiosensitivity of chromosomes of human PBL.

**Ascorbic acid.** The most common antioxidants include AA, which is designated as the "signal molecule that causes specific activity in cells" [37]. Several studies have revealed the ambiguous nature of AA action in human cells. It is found that, unlike the animals, human organism does not produce AA and its nutritional deficiency promotes most cancers of the stomach, esophagus, oral cavity, and cervix [38]. Nobel Prize winner L. Pauling proposed the idea of prevention and treatment of neoplasia with high doses of AA (10 g per day). The encouraging results were obtained, but the general effect of clinical testing in the most cases turned out to be misleading or even absent. Research efforts by increasing the dose up to 100 g AA per day did not cause intoxication, but had no result either [39]. There is a contrary view, according to which vitamins, including AA, are inappropriate to use as a prophylactic measure to reduce cancer risk [40]. The data on antimutagenic properties of AA are not always confirmed, even in methodologically similar studies.

We have studied the features of the modification of radiation-induced cytogenetic effects in cultured human PBL under the influence of a potential co-mutagen AA [41]. It has been shown that at a range of concentrations from 20.0 to 80.0 g/ml AA did not affect the level of spontaneous chromosomal aberrations in lymphocytes of donors, which corresponded to mean population values (2.0 ± 0.86/100 metaphases). The results are consistent with the data on the absence of the effects of antioxidant vitamins on the spontaneous mutation level in human lymphocytes [42].

Analysis of the frequency of chromosomal damage induced by irradiation of PBL in G0-phase of cell cycle (at a dose range of 0.3–2.0 Gy) with or without post-irradiation exposure to AA at therapeutic concentrations of 20.0 µg/ml of blood showed that radiomodifying effect of the drug is not clear. When combined with the low dose X-ray exposure (0.3 Gy), AA at therapeutic concentrations of 20.0 µg/ml of blood caused 1.5-fold decrease of the total frequency of chromosomal aberrations compared to the effect of irradiation only. This is consistent with the findings of the study [43] showing that AA at therapeutic doses exhibits radioprotective activity by utilizing free radicals and improves the antioxidant status of the cells. When irradiating PBL at a relatively high dose (2.0 Gy) under the influence of AA at the same therapeutic concentration, we have detected a radiation-potentiating effect, i.e. nearly 1.4-fold increased total frequency of chromosomal aberrations, indicating the co-mutagenic properties of the drug. The observed enhancement of radiation-induced cytogenetic effect is due to dicentric chromosome formation. Since the formation of dicentrics requires local double chromosome breaks resulting from irradiation, the increased yield of aberrations of this type under the additional action of AA can be interpreted as a proof of the strengthening of the primary radiation damage under the influence of the studied drug. These effects can identify the problem of clastogenic action of AA on the human genome [44].

Special attention is given to the modification of cytogenetic effects of low dose IR by AA at concentrations exceeding the therapeutic range. It was found that an additional post-irradiation effect of AA at the doses 40.0–80.0 µg/ml of blood increased the overall frequency of chromosomal aberrations by 1.2 and
1.4 times, respectively, when compared to the effect of irradiation only at the dose of 0.3 Gy. It may also indicate a co-mutagenic activity of AA, but at the range of concentrations that exceeds the therapeutic value by 2 and 4 times. Since the low dose irradiation along with chromosomal breaks can also induce their potential pre-mutational changes, the supplementary potentiating action of co-mutagens at a high concentration range may contribute to their impact into the structural chromosomal rearrangements, including the suppression of DNA repair enzymes [45].

Caffeine. According to the study [29], a purine compound caffeine activates the cellular mechanisms of post-radiation recovery and DNA repair. The results of our study are in disagreement with this conclusion. We have shown that the post-irradiation effect of caffeine (200 µg/ml of blood) during 2 h increased the frequency of chromosomal aberrations in donor’s PBL irradiated in vitro at low doses by 5.5 times as compared with irradiation effect only. This increase was due to chromosomal aberrations, mostly paired fragments and dicentrics. Caffeine alone had slightly increased the spontaneous level of chromosomal aberrations. An explanation may be the following. By blocking the entry of cells into the DNA synthesis and mitosis, irradiation allows cells to recover from radiation-induced damage. Caffeine shortens the duration of such blocking, and the cells enter mitosis with unrepaired injuries, so the yield of double-strand DNA breaks and chromosomal aberrations increases. We obtained the data at the chromosomal level using human somatic cells irradiated at low doses, showing the co-mutagenic effect of caffeine. These results are in contradiction to the data of study [29], possibly, due to the different concentrations of the drug and radiation doses used. Nevertheless, it should be noted that the combined action of radiation and chemicals could often cause a negative synergistic effect.

CONCLUDING REMARKS
Currently, a lot of attention should be given to the assessment of mutational rate and cancer risk in human population exposed to low dose IR. This requires further study and use of modifiers, selective effects of which enhance the radiosensitivity of the human genome and, thus, reduce cancer risk. The prediction of modification of low dose IR effects is complicated by a number of the following circumstances.

i. It’s necessary to account the individual radiosensitivity of the human body. If the intensity of the radiation exposure is high, the individual characteristics of a person are not of pivotal role, since the volume of damage exceeds the protective and compensatory capacities of the organism. Therefore, the assessment of individual radiosensitivity is particularly important at the range of low dose IR responsible for the formation of stochastic effects. Therefore, we recommend for the use the chromosomal G2-test modified on the basis of the classic radiation cytogenetics [46].

ii. The modification of the low dose IR effects at the cellular level is weakly dependent on cell-cycle phase. This could be explained by the fact that under the influence of low dose IR, the differences between the degrees of cell radiosensitivity during cell cycle are balanced.

iii. The modification of low dose IR effects significantly depends on the relative biological effectiveness (RBE) of IR. It is known that this variable is introduced in order to compare the biological effects of the action of various types of radiation at the equal absorbed dose. We have found [32] that the largest RBE value of densely IR is observed at low doses. For example, the RBE coefficient of fast neutrons in this dosage range may reach a value of 10 and more. Therefore, a targeted modification (enhancement/attenuation) of induced effects will be more pronounced.

iv. The health of the individual should be taken into account. Thus, the progression of tumor growth is associated with the emergence of new mutational events due to the inhibition of reparative processes because of immunosuppression. Therefore, non-malignant cells (e.g. blood cells) of cancer patients are characterized by high radiation sensitivity, which affects the modification effects of low dose irradiation.

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